The appetizing and satiating effects of odours

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Thesis

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Summary

Background and aim

Unhealthy eating habits such as unhealthy food choices or overeating increase the prevalence of obesity, diabetes, cancer, cardiovascular and other diseases. Therefore, it is important to understand how separate factors, such as sensory processes, influence our eating behaviour. As one of the sensory modalities, olfaction has a relationship with food intake regulation. Previous research reveals that food odours can induce both appetite and satiation. In this thesis, we split appetite and satiation into a 'general' part and a 'food specific' part. General appetite and general satiation refer to the desire to eat in general. General satiation measured by subjective ratings (e.g. by using line scales) is also named 'subjective satiation'. The specific part refers to the desire to eat a specific food: e.g. the appetite for a banana or the appetite for tomato soup.

The main objective of this thesis was to investigate under which circumstances odours are appetizing or satiating in order to identify factors that influence our eating behaviour. Odours arrive at the odour receptors via two routes: the orthonasal route via the nose to perceive the outside world or retronasally via the mouth to 'taste' the food. The appetizing and satiating effects of ortho- and retronasally smelled odours were investigated by varying the **odour exposure time**, the **odour concentration** (retronasal only), the **odour type**, passive versus active sniffing (orthonasal only) and by **switching between odour types**.

Methods

We conducted six within-subject experiments. All participants were healthy normal-weight women (age 18-45 y and BMI 18.5-26 kg/m^2). In four experiments (studies 2A, 2B, 3A and 3B), we investigated the appetizing and satiating effects of orthonasal odours, with two experiments addressing odours that were smelled passively in rooms with ambient odours (chapter 2) and two addressing actively smelled odours by sniffing the contents of a cup (chapter 3). In studies 2A (passive, n=21), 2B (passive, n=13) and 3A (active, n=61), we investigated the effects of **exposure time** and **odour type** on appetite, the appetite for specific foods, food preference and food intake. Differences between **passive** and **active exposure** were investigated by comparing the data from 2A and 3A**.** In the fourth experiment (n=30) using a similar set-up, sweet and savoury odours were presented directly after each other, to explore the effects of daily encounters with a variety of food

odours (i.e. **switching**). In all orthonasal studies, general appetite and the appetite for specific foods were monitored over time, using visual analogue scales. General appetite comprised hunger and desire-to-eat ratings. The appetite for specific products addressed the appetite for smelled products and the appetites for a set of other products that were congruent and incongruent with the odour (studies 2A, 2B, 3A and 3B). Food preference was assessed using a computerised program offering pairs of food pictures (studies 2A, 2B and 3B).

Furthermore, two experiments addressed the satiating effects of retronasal odours while consuming tomato soup *ad libitum* (studies 4A and 4B). The retronasal odour exposure was disconnected from the soup base consumption by use of a retronasal tube that was connected to an olfactometer. The odours were delivered directly into the nasal cavity at the moment a sip of soup base was swallowed. In study 4A (n=38), the satiating effects of **odour exposure time** (3 and 18 s) and **odour concentration** (5x difference) were investigated. In study 4B (n=42), we investigated whether addition of **cream odour** to tomato soup, in combination with a low or high viscosity, affected satiation. Hunger and appetite ratings were monitored over time during odour exposure, by using 100 mm visual analogue scales (VAS).

Results

The results showed that **orthonasal** exposure to food odours influenced the appetite for specific foods via a **typical pattern**: the appetite ratings for the smelled foods increased by **+6-20 mm** (SSA; all $P<0.001$), the appetite for congruent sweet and savoury foods increased by **+5 mm** and the appetite for incongruent sweet and savoury foods decreased by **-5 mm** (all $P<0.01$), measured by using 100 mm VAS (studies 2A, 2B, 3A and 3B). This typical pattern was found in all studies, independently of passive or active smelling, exposure time or switching between odours (studies 2A, 2B, 3A and 3B). Results in study 3B showed that the appetite for specific products adjusted to the new odour within one minute after a switch between sweet and savoury odours. Similar results were found with a computerised food preference program, in which participants chose repeatedly between pairs of foods (studies 2A, 2B and 3B). Food preference shifted in circa 20% of the choices. Furthermore, passively smelled food odours had a large effect on the appetite for the smelled foods (+**15 mm;** $P<0.001$) and a small effect on general appetite (+**4 mm**; $P=0.01$; study 2A). Actively smelled food odours had **no** significant effect on general appetite or food intake (studies 3A and 3B). Non-food odours appeared to suppress general appetite slightly (**-2 mm,** $P=0.01$). The appetizing effects did **not change over time** during a twenty-minute odour exposure (studies 2A, 2B, 3A and 3B) and the typical pattern of odour effects on the appetite for specific foods was not affected by switching between sweet

and savoury odours (study 3B). The pleasantness of the odour decreased by -**4 mm** during active smelling (P=0.005), whereas the appetite for the smelled food remained high (P<0.001; study 3B).

Furthermore, the results from the **retronasal** studies showed that an increase in both retronasal odour exposure time and concentration reduced *ad libitum* intake by **9 %** (i.e. 3 sips and 22 kJ; P=0.04) and had no effect on subjective satiation (study 4A). Adding cream odour decreased subjective satiation with circa **5 %** between 7 and 13 minutes after the start of consumption (P=0.009), but did not affect *ad libitum* intake (study 4B). Retronasally smelled odour significantly contributed to the development of sensory-specific satiety (study 4A).

Conclusions

Orthonasally smelled odours affect to a larger extend what you eat, than how much you eat. They influence the appetite for specific foods via a typical pattern: the appetite for the smelled foods and for congruent sweet or savoury foods increases, whereas the appetite for incongruent sweet or savoury foods decreases. This typical pattern is **independent of exposure time, passive or active smelling and switching** between odours. The reason for this pattern is unknown, however, it may be caused by the preparation of the body for the intake of the smelled food, as food odours may provide information about the nutrient composition of their associated foods. Furthermore, **passive** odour exposure may enhance general appetite (how much), whereas **active** smelling appears to have no effect. Interestingly, the appetite for the smelled foods remained elevated during the 20-minute smelling, although the pleasantness of the smelled odour decreased a little over time. This shows an earlier assumption from literature incorrect: a decrease in pleasantness of the odour does not lead to less appetite for the smelled food. This seeming contradiction may result from different mechanisms, such as a decrease in hedonic value during prolonged sensory stimulation on the one hand and anticipation of food intake on the other hand. Furthermore, food odours were found to change preference in circa 20% of the cases. Probably, food odours shift food preference, but do not overrule strong initial preferences in circa 80% of the cases.

Moreover, **retronasally** smelled odours probably have a small influence on satiation, though the evidence is not very strong. An increase in both retronasal **odour concentration** and **odour exposure time** may enhance satiation. Adding **cream odour** may temporarily affect subjective satiation but does not affect food intake. However, the satiating effects that were found in these studies with retronasal odour exposure were

borderline significant and data on food intake and subjective appetite ratings were not consistent, which probably reflects the small effect size.

Orthonasal odours influence food preference and could potentially be used to encourage healthy eating behaviour. The studies in this thesis were conducted under controlled circumstances and the results possibly deviate from behaviour in daily life. Therefore, it is unclear how strong the influence of odours is on our eating behaviour in daily situations. Finally, we advise product developers not to focus on changing retronasal odour characteristics in order to enhance satiation of products, seen the small effects that were found in this thesis.

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Chapter 1

General introduction

Introduction

Humans evolved to eat when foods are available as a buffer against future food shortages.[1] Our reward system in the brain, consisting of liking, wanting and learning, produces 'go' signals for consumption that can be weakened by satiety, but never generates a strong 'stop' signal.[2] Therefore, the current obesogenic food environment, where high caloric foods are often available encourages unhealthy eating habits.[3] Unhealthy eating habits such as unhealthy food choices and overeating increase the prevalence of several diseases, e.g. obesity, diabetes, impaired cognitive function and cancer.[1, 4, 5] It is therefore important to understand which factors influence eating behaviour. In our modern society, humans are able to choose from a wide range of foods varying in physical/chemical properties, nutrient composition, energy density, palatability and sensory characteristics. Sensory processes play a key role in the selection of foods and determine meal size and the enjoyment of eating foods.[6-8] For instance, an increase in the extent of sensory exposure was found to reduce food intake.[9, 10] Another example is sensory-specific satiation (SSS), which is the decrease in reward of eaten foods, relative to foods that were not eaten.[11 12] SSS, originally named sensory-specific satiety, is suggested to drive the ingestion of a variety of foods.[11, 13]

As one of the sensory modalities, olfaction has a close relationship with eating behaviour.[14] The research described in this thesis investigated the appetizing and satiating effects of odours. These effects were followed over time, as appetite and satiation are dynamic processes. This chapter starts with a general explanation of eating behaviour, followed by an introduction of several important aspects of olfaction, in order to be able to understand the effect of odours on appetite and satiation. Subsequently, the odour characteristics that potentially influence appetite and satiation will be explained in more detail. Finally, the aim and thesis outline will be presented. An overview of important abbreviations and definitions is given in the glossary.

Eating behaviour

Eating behaviour consists of the food choices that people make (WHAT), the amount they eat (HOW MUCH) and the timing of meal and snack intake (WHEN). A brief and non-exhaustive overview of the factors that influence eating behaviour before, during and after food intake is given in this chapter.

Metabolic signals influence eating behaviour

Eating behaviour is regulated to a large extent by brain processes that underlie the perception of food flavours and the desire-to-eat those foods[14] in combination with brain processes that respond to physiological signals from the gastrointestinal tract and adipose tissue that reflect the nutritional status of the body.[15] The adipose tissue communicates the fat storage in the body to the brain by releasing the hormone leptin, which is an example of long-term food intake regulation.[16] On the short-term, food intake is regulated by several physiological processes, such as signals from the stomach and the release of satiety hormones from the gastrointestinal tract.[15] The rise of the level of the 'hunger hormone' ghrelin at fixed times just before a meal is thought to play a role in the anticipation of food intake and meal initiation.[17, 18] Adipose tissue also influences the ghrelin level for the long-term regulation, revealing the complexity of the system.[19] During and after a meal, metabolites such as glucose, amino acids and free-fatty acids enter the blood stream and affect the release of peptide hormones such as cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1) that signal the brain and consequently suppress hunger.[20] All these processes together influence the extent of hunger, appetite, satiation and satiety.[21]

Important terminology

Hunger reflects the physiological need for food. Appetite on the other hand, is referred to as hedonic hunger[22] and incorporates eating in the absence of hunger and is influenced by the environment, for example by food cues. In this thesis we distinguish between **general appetite** that is independent of the specific food and **sensory-specific appetite (SSA)** that is the increase in appetite specific for the cued food. SSA is in this thesis defined as the opposite of SSS. A meal is terminated when the inhibitory factors become larger than the promoting factors.[23-25] Satiation refers to the processes that bring a meal to an end[26] and is considered the feeling of fullness during a meal. Satiation in this thesis is referred to as **general satiation**. Satiety is the feeling of fullness after a meal, influencing the time until the next meal. **Sensory-specific satiation (SSS)** is the decrease in pleasantness or desire-to-eat of eaten foods, relative to foods that were not eaten.[11, 12] After a meal, hunger is low and satiety is high (see glossary for definitions).

Sensory processes influence eating behaviour

Sensory processes in combination with metabolic factors steer brain processes that determine eating behaviour, such as food selection.[27] During daily exposures to foods, associations between the nutrient composition and the sensory properties of foods are formed.[28] This flavour-nutrient learning affects the pleasure that is derived from foods,[29, 30] which subsequently influences food selection. These associations also aid in food

selection in case of nutrient deficits[31, 32] or facilitate the selection of a variety of foods. Furthermore, external factors, such as food cues change food preference.[33-37]

Sight, smell and taste are food cues that increase the appetite for the cued food,[36-41] which we defined as sensory-specific appetite (SSA) in this thesis. Additionally, exposure to the smell and taste of pizza increased the desire-to-eat 'pasta and tomato sauce', 'chicken tikka masala' and 'scrambled egg, chips and baked beans', besides an increase in pizza.[37] It is thought that food intake can be larger after exposure to food cues,[42, 43] although results from human studies on food intake are not consistent.[33, 37, 38, 44-47] Exposure to food cues namely induce cephalic phase responses, which are physiological processes that prepare the body for food intake.[42, 48] Food intake poses a challenge on the maintenance of homeostasis and keep balance in the osmotic values in the body.[42] In anticipation of food intake, the cephalic phase responses help to process the inflow of nutrients and improve the digestion, absorption and use of nutrients, for example by temporarily dropping the blood glucose level.[49] These responses consist of hormone releases, changes in glucose levels and gastric activity, increase in heart rate and blood pressure, salivation, and more.[43] The cephalic phase responses that are elicited after exposure to food cues are weak compared with the physiological responses during food intake.[48]

Sensory processes are also important during eating. For instance, digestion of food is less optimal in patients with a stomach-tube who lack sensory exposure during food intake.[50] In addition, people eat less when oral exposure time is longer.[8, 51-55] Wijlens *et al.*[8] demonstrated that oral exposure time was at least as important in decreasing energy intake as gastric filling volume. Cecil *et al.*[56] found that eating high-fat soup suppressed hunger, induced fullness and reduced energy intake, compared with eating iso-caloric high-carbohydrate foods, but not when these soups were infused intra-gastrically,[56] which emphasizes the importance of sensory exposure. Furthermore, we would like to add that SSS plays a role in eating behaviour during and just after a meal. Development of SSS was found for the taste, smell, feel (texture) and sight (colour) of eaten foods.[11, 12, 57-59] SSS should not be confused with alliesthesia, which is the difference in pleasantness of the same food caused by differences in hunger.[60] In general, foods are better liked when hungry than when satiated.

Other factors that influence eating behaviour

Besides food cues, there are many other external factors that influence eating behaviour, such as time of day, food availability, distraction, music, colours, temperature and the company with whom is eaten (for review see e.g.[61]). People tend to consume faster when hearing fast music,[62] eat more when distracted[63] and eat less when we are alone.[64] Ice

cream is more attractive when the temperature is high and especially women tend to copy their eating behaviour from other women.[65, 66] Plate size and portion size also affect how much we eat.[67] According to Wansink,[67] the external factors that affect how much we eat either inhibit monitoring the actual food intake or set consumption norms. Furthermore, individual differences such as character, BMI, gender, age and culture largely influence eating behaviour, as well as the thoughts, beliefs[68] and expectations[69] that we have about foods and physical activity, habits, emotions[70] and diet.[71] The diet that was eaten in the past affects the present set point for metabolic balance.[71] A Western diet that is high in fat and sugar content affects brain responses or even damages the brain[72] and has a negative impact on weight control.[73] Even the diet our mothers had during pregnancy influences our current eating behaviour.[74]

Link between olfaction and appetite / satiation

Olfaction may have a close relationship with eating behaviour.[75] There are indications that olfaction is interrelated with the hormones of energy homeostasis. Ghrelin, leptin,[76-78] adiponecting,[79] cholecystokinin,[80] Neuropeptide-Y[80] and glucagon-like peptide-1,[80] which are all satiety hormones, bind to receptors in different layers of the olfactory systems in rats and mice. If and how these hormones modulate olfaction or vice versa in humans remains to be established.[80] However, it is known that hunger selectively biases attention toward food cues such as odours[81] and increases food cue reactivity.[81-84]

Olfaction

What and how much we eat is strongly influenced by sensory processes, among other factors. The smell, taste and trigeminal stimuli together, also called the chemical senses, determine the flavour of a food.[85, 86] In this thesis, the role of smell in eating behaviour is further explored. Current knowledge on how odours are perceived and the factors that influence odour perception are explained in this section, in order to better understand the relationship between odours and eating behaviour.

Dual sense

Olfaction is the sense of smell. Odours are perceived via two routes: orthonasal and retronasal. Therefore, olfaction is called a dual sense (Figure 1.1). Orthonasal odours are smelled through the nose and give information about the external world. Retronasal odours reach the olfactory epithelium after passing through the pharynx during swallowing of foods and provide information about foods. The odours originate from the food in the mouth and are perceived as if they are tasted.[87, 88] The same odorant sometimes smells different when perceived via the orthonasal or the retronasal route (see

[89] for a review). Such difference may be caused by the differences in air flow that influences the absorption of odorants across the mucosa.[90-92] How odours are detected is explained in the next section.

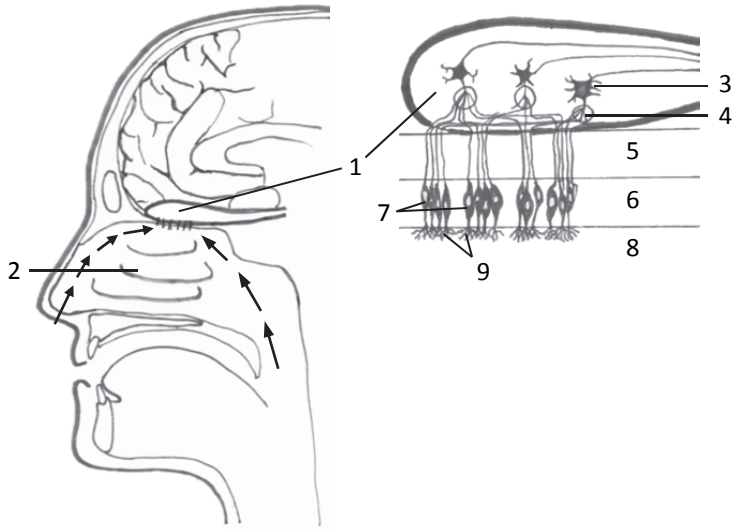


Figure 1.1 Human olfactory system**.** 1. Olfactory bulb; 2. Turbinate's; 3. Mitral cells; 4. Glomerulus; 5. Bone; 6. Olfactory epithelium; 7. Olfactory receptor neurons; 8. Mucosa; 9. Cilia. The arrows represent the orthonasal and retronasal routes.

The olfactory system

Air that enters the nose circulates around three turbinate's, which humidifies and warms up the air. Odorant binding proteins probably aid the transport of odorants to the long cilia of **olfactory receptor neurons** (Figure 1.1).[93, 94] **Cilia** are thin structures in the mucosa of the olfactory epithelium and contain odour receptors. Odorants also diffuse in the **mucosa** by themselves. A typical human nose contains around 40 to 50 million olfactory receptor neurons, coded by circa 350 genes (900, but many are non-functional).[95] In comparison, a dog has 10 to 20 times more olfactory receptor neurons.[96] The axons of the olfactory receptor neurons group into olfactory nerves and project to the **olfactory bulb** in the brain, which contains **glomeruli** and mitral cells. Each **mitral cell** synapses in the glomeruli with around 1000 olfactory neurons, which is an enormous convergence of information at this point.[97] From the olfactory bulb, the olfactory information is transported to a number of other brain areas, including the piriform cortex, which is important for odour identification.[98, 99] The sensory signals are linked with reward and motivation in the orbitofrontal cortex, a higher cortical area, where also SSS can be found.[100] One of the challenges of olfaction is the recognition of complex chemical blends against a noisy background.[101]

Odour identification and intensity

Literature typically reported that humans are able to detect around 10.000 different odours,[102] however, recently this number has been increased to more than one trillion.[103] The olfactory nerves and the mitral cells are little specific and respond to many different odorants.[104] However, the unique pattern of neurons in the olfactory bulb that are excited by an individual odour, including the time of excitation, is called an 'odour image' and probably serves as a code to identify odours and its intensities.[14, 105, 106]

Odour identification and intensity perception is complicated by the large moment-to-moment variability in the number of odorants that actually bind to the olfactory receptors.[107] The number depends on odour concentration, breathing pattern (sniffing), phase in the breathing cycle, the anatomy of the nose and a stuffed up nose.[108] A cold may block the air stream to the olfactory epithelium, thereby diminishing the olfactory exposure.[108] Sniffing increases the number of odorants that reach the olfactory epithelium, which increases the intensity[109] and therefore the ability to identify an odour. Identification is not possible near detection threshold.[110] The intensity range of odours is not very wide. An odour concentration of 10-50 times the concentration at detection level, already reached the maximum intensity.[111] Doubling the concentration increases the intensity with circa 50%.[108] Therefore, it can be suggested that detection of odorants is more important than quantification of the odorants. Additionally, the perceived intensity of orthonasal odours is in most cases higher than that of retronasal odours.[112]

Furthermore, odour intensity drops to around 30% of its original intensity during odour exposure,[113] due to the development of adaptation in the brain. Adaption often impairs the perception of a weak odour after smelling a stronger odour. Therefore, it is important to take the occurrence of adaptation into account when setting up odour experiments. For instance, by keeping the amount of odour exposure per participant low, giving enough time in between odour exposures and preferably test weak odours first.

Cross modal interactions

Odour and flavour perception are strongly influenced by input from other sensory modalities. Smelling bananas often accompanies seeing bananas and eating bananas accompanies taste and smell. The brain integrates these sensory signals from different modalities into a new perception that is different from the perception of the separate modalities.[85, 114, 115] Examples of this cross-modal integration are the sweeter taste of a solution that is combined with a congruent odour[85, 114, 116] and the increase in perceived thickness upon perception of retronasal cream odour.[117] Furthermore, pleasantness of a

flavour can be much higher than the pleasantness of the separate taste and odour[118] and depend on the congruency between the taste and the odour.[116]

Odour characteristics that affect appetite and satiation

In most research, a combination of sensory modalities was investigated in relation to appetite and satiation, leaving the unimodal effects of odour uncertain. Both orthonasal and retronasal odours are suggested to generate both appetizing and satiating responses.[35, 119] The appetizing effects of orthonasal odours are well-known, such as increases in hunger, cravings and food intake.[14, 35, 36, 120] On the other hand, a few studies indicate that orthonasal odours have satiating effects and decrease hunger,[121] food intake[44, 46] and olfactory SSS.[57] Also retronasal odours are involved in the preparation of the body for food intake when appetizers are consumed (amuse). So far, the few studies that investigated the satiating possibilities of retronasal odours found small changes in rated satiation.[119, 122, 123] Based on literature, several factors were identified that possibly affect the appetizing and satiating effects of odours: route of odour perception, odour type, odour exposure time, odour concentration, sniffing behaviour and switching between odour types. Each factor is explained below.

The **route** of odour perception, orthonasal versus retronasal, determines how odours are processed in the brain,[89, 124, 125] producing separate odour images that interact with other brain areas[14] and have distinct effects on cross-modal integration[14, 117] and odour intensity.[126-128] Bult *et al.*[117] for example showed that providing cream odour retronasally increased the perception of thickness and creaminess, while orthonasal cream odour did not. Interestingly, it was found that orthonasal and retronasal olfaction represent qualitatively distinct sensory experiences,[125, 129] and it has been hypothesised that orthonasal perception is associated with the anticipatory phase in food reward, and retronasal perception with the receipt of food.[119, 124]

Furthermore, **odour type** may affect appetite and satiation via associations with foods that differ in their satiating capacity and macronutrient composition. Several studies investigated the effects of adding odour to foods, which results in retronasal exposure. A recent study reported that adding the odour of olive oil to plain yoghurt increased satiety.[130] The investigators compared the weight gain and serotonin level of participants who daily consumed 500 g of either plain yoghurt or yoghurt enriched with the odour of olive oil over a 3 month period. The yoghurt was given as a supplement to their normal diet. Participants who consumed plain yogurt consumed more of the other foods than the participants who consumed yoghurt with the olive oil odour. Furthermore, the satiety

hormone serotonin was greater in the olive oil group, than in the plain yoghurt group. Another study showed that adding olive oil extract, without the fat, increased blood flow in the taste cortex, which might simulate fat perception.[131] Similarly, Ruijschop *et al.*[119] reported that participants felt more satiated after consumption of yoghurt that was enriched with a flavour that cued for proteins or carbohydrates, than normal yoghurt. However, the addition of lactones, supposed to cue for fat, had no effect.[119] Additionally, Ruijschop *et al.*[122] reported an increase in subjective satiation after an increase in retronasal odour complexity. Altogether, there are indications that retronasal odour type affects satiation. Orthonasal odour type may also affect appetite and satiation via their associations with the nutrient composition of foods. Food odours were found in general to enhance appetite,[36, 120] whereas non-food odours supressed appetite.[132, 133] Besides via the association with the nutrient composition of foods, odours may also influence the activity of the autonomic nervous system and therefore food intake.[134] In a long term study, rats were three times per week for 15 minutes exposed to grapefruit oil odour or limonene odour, which reduced food intake and body weight,[135, 136] but a 15-minute daily exposure to lavender oil odour and linalool odour increased food intake and body weight.[137] Therefore, odour type may influence the relation between odours and appetite/satiation. The short-term effect of odour type has so far not been investigated in a single study.

Both orthonasal and retronasal odour **exposure time** potentially affect appetite and satiation. Participants felt more satiated after longer retronasal odour exposure than after shorter exposure per sip,[123] which is in line with the many studies showing an increase in satiation after longer sensory exposure time.[52, 54, 138, 139] Furthermore, we observed that all studies with a brief exposure to food cues report an appetizing response,[37, 38, 140] whereas studies that report a satiating response to food cues all expose their participants for at least five minutes to the food cue,[57, 121, 141-143] which leads to the suggestion that exposure time is important for the differences in appetizing or satiating response to food cues and thus orthonasal odours.

Additionally, a small effect of tastant **concentration** on food intake was found[55, 144] and it may be hypothesised that a similar relationship is valid for retronasal olfaction. As far as we know, a unimodal effect of odour concentration on appetite or satiation has never been investigated for neither orthonasal nor retronasal odours.

Furthermore, **sniffing** in humans and other animals is considered a means of exploring the environment when motivated to consume.[145] When hungry, humans have longer sniffs with a greater amplitude.[145] In addition, sniffing affects the odour concentration near the olfactory epithelium and may also attract attention toward the odours. Therefore, sniffing behaviour, i.e**. passive** smelling or **active** sniffing, may modulate odour induced appetite or satiation.

Finally, **previous exposures** to food cues possibly prime a body and may therefore interfere with new exposures. As far as we know a **switch** in food cue exposure was never investigated before.

During odour exposure, associations are formed with the food, context, emotions etc. that shape the perception of future exposures to food and its odours. These associations affect eating behaviour during present odour exposures (visualised in Figure 1.2).

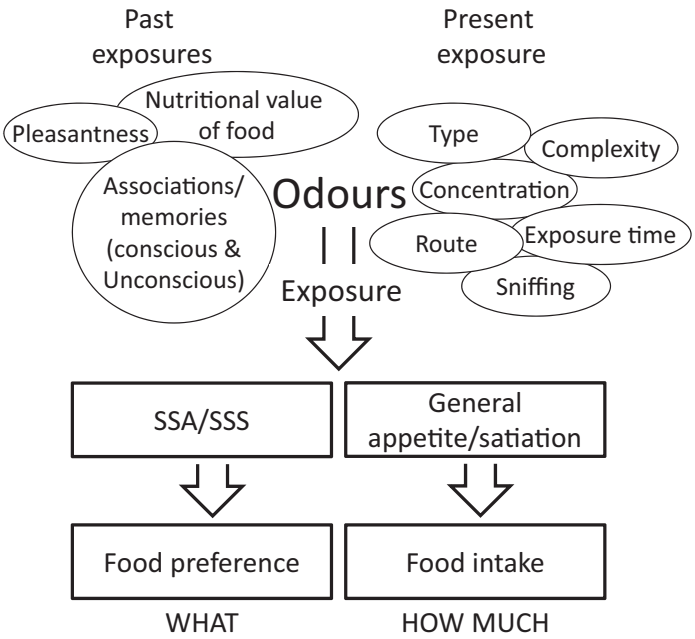


Figure 1.2 Conceptual model about the influence of odours on what and how much we eat. This concept applies to an eating situation in which there is a variety of foods.

Aim and thesis outline

Orthonasal odours potentially enhance both appetite and satiation. Retronasal odours during food intake were found to influence satiation. The exact odour characteristics and circumstances under which odours are appetizing or satiating need clarification before results can be used for e.g. product application or dieting programs. This was further investigated in the present thesis. The main objective of this thesis was to investigate under which circumstances odours are appetizing or satiating in order to identify factors that influence our eating behaviour, i.e. what and how much is eaten.

The factors that potentially influence the appetizing and satiating effects were investigated in separate studies: odour **exposure time** (orthonasal: chapters 2A, 2B, 3A; retronasal: chapter 4A), odour **concentration** (retronasal: chapter 4A), odour **type** (orthonasal: chapter 2A; retronasal: chapter 4B), **active versus passive** exposure (orthonasal: chapters 2A en 3A) and **switching** between odours (orthonasal: chapter 3B).

In chapters **2 and 3**, we investigated the satiating effects of **orthonasal** odours. In **study 2A**, we investigated the effect of **passive** odour exposure on general appetite and SSA over time. The odours were spread in a room. General appetite and sensory-specific appetite were monitored over time to investigate if effects of odours shift. We hypothesised that a one-minute exposure to food odours increases general appetite, whereas a 10-20 minute exposure decreases general appetite. Furthermore, eight food and non-food odours were used, to explore potential differences on appetite, SSA and food preference. Simultaneously, another group of participants was exposed for either one minute or twenty minutes to a food odour in **study 2B**, to specifically investigate the effect of exposure time on food preference. In **study 3A**, we used a similar set-up as in study 2A, but changed to **active** odour exposure, using two types of banana odour and a control condition. The odours were presented in a cup that was covered with a tissue and perceived via active sniffing. Possible differences in appetite and SSA due to passive vs active sniffing could be explored by comparing the data from study 2A and 3A. In addition, *ad libitum* intake was measured to include a behavioural measure for satiation. In **study 3B**, the participants also actively sniffed food odours, but this time the separate odours were presented directly after each other, to investigate the influence of previous odour exposures (i.e. switching) on appetite and food preference.

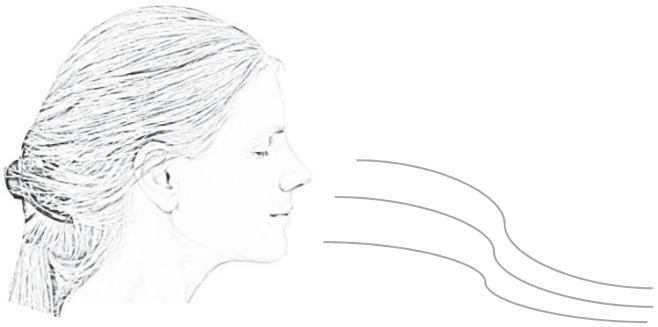
In **chapter 4**, we investigated the satiating effects of **retronasal** odours. The retronasal odours were presented in the nose via a retronasal tube that was attached to an olfactometer, to investigate the unimodal effect of odours on satiation. In **study 4A,** four

odour release profiles, differing in odour exposure time and odour concentration were generated using the olfactometer and their different effects on *ad libitum* intake and subjective appetite ratings investigated**.** We hypothesised that an increase in retronasal odour exposure time and concentration leads to an increase in satiation. In **study 4B**, the influences of cream odour and viscosity on satiation was assessed. We hypothesised that the addition of cream odour and an increase in the viscosity of soup both lead to an increase in satiation and a decrease in food intake, because they may both be associated with an increase in energy density. In **chapter 5**, the general discussion, the main results and methodological aspects are discussed and directions for further research suggested.

In this thesis, we consider both subjective appetite ratings and *ad libitum* intake as measures for appetite and satiation (how much), bearing in mind that a change in subjective ratings does not automatically mean a change in how much is eaten.[21, 146] SSA, SSS and food preference are considered to measure what is eaten (Figure 1.2), with food preference being the result of choices between sets of foods.

Chapter 2A

Orthonasal passive odour exposure



Odours: appetizing or satiating? Development of appetite during odour exposure over time

Based on 'Odors: appetizing or satiating? Development of appetite during odour exposure over time.' *International Journal of Obesity,* 2014; **38**(5). Mariëlle Ramaekers, Sanne Boesveldt, Catriona Lakemond, Martinus van Boekel, Pieternel Luning.

Abstract

Background: Exposure to palatable food odours influences appetite responses, either promoting or inhibiting food intake. Possibly, food odours are appetizing after a short exposure (of circa 1-3 minutes), but become satiating over time (circa 10-20 minutes).

Objective: To investigate the effect of odour exposure on general appetite and sensory-specific appetite (SSA) over time.

Design: In a cross-over study, 21 unrestrained women (age: 18-45 y; BMI: 18.5-25 kg/m^2) were exposed for 20 minutes to eight different odour types: five food odours, two non-food odours and no-odour. All odours were distributed in a test room at supra-threshold levels. General appetite, SSA and salivation were measured over time.

Results: All food odours significantly increased general appetite and SSA, compared with the no-odour condition. The non-food odours decreased general appetite. All effects did not change over time during odour exposure. Savoury odours increased the appetite for savoury foods, but decreased appetite for sweet foods, and vice versa after exposure to sweet odours. Neither food odours nor non-food odours affected salivation.

Conclusions: Palatable food odours were appetizing during and after odour exposure and did not become satiating over a 20 minute period. Food odours had a large impact on SSA and a small impact on general appetite. Moreover, exposure to food odours increased the appetite for congruent foods, but decreased the appetite for incongruent foods. It may be hypothesised that, once the body is prepared for intake of a certain food with a particular macronutrient composition, it is unfavourable to consume foods that are very different from the cued food.

Keywords: Appetite, Food choice, Odour type, Olfactory cue, Sensory-specific appetite, Sensory-specific satiety

Introduction

Physiological signals that reflect the nutritional status determine how hungry or satiated we are. Satiety determines the duration till the next meal, whereas hunger generates appetite. Appetite, however, refers also to eating in the absence of hunger and is co-regulated by many other internal and external parameters, for example, food cues.[147] Exposure to palatable food cues promotes food intake,[36, 38, 140, 148] but may also inhibit[44] food intake. In view of the obesity epidemic, it is important to understand which factors determine the appetizing and/or satiating responses to food cues. In the present study, two factors were investigated: odour exposure time and odour type.

Exposure to food cues, for example, the sight, smell or taste of freshly baked bread, can enhance appetite and salivation.[33-38, 48, 61, 149] This 'appetizer effect' results from the anticipation of ingesting food and optimizes digestion, absorption and use of nutrients by means of physiological responses, called cephalic phase responses.[42, 48] Moreover, exposure to food cues specifically increases the appetite for the cued food relative to the appetite for other foods,[36-38] further on referred to as 'sensory-specific appetite' (SSA). For example, the appetite for pizza increased more than the appetite for other foods, after one minute exposure to the sight and smell of pizza.[37, 38] Most literature investigated the effect of a combination of food cues on appetite, but some showed that odours alone are able to enhance both hunger and salivation[35] and thus are appetizing.

On the other hand, there are indications that odours can be satiating as well after a longer exposure time.[44, 57, 150-152] Jansen *et al.*[44] showed that normal-weight children decreased their intake of palatable sweet and savoury snacks after smelling those foods for 10 minutes, compared with no smelling. In addition, Rolls and Rolls[57] found that smelling bananas or chicken for 5 minutes decreased the pleasantness of the smell of bananas and chicken, respectively relative to the pleasantness of other foods that were not smelled. Both Jansen *et al.*[44] and Rolls and Rolls[57] attributed their results to sensory-specific satiety (SSS). SSS was defined as a larger decrease in pleasantness of eaten foods relative to the decrease in pleasantness of uneaten foods[12] and is the opposite of SSA. Exposure to food cues for a longer time without the ingestion of food by modified sham feeding (chewing foods without swallowing) increased both SSS[57, 141, 142] and metabolic satiety.[152] We hypothesised that food odours are appetizing after a short exposure (of circa 1–3 minutes), but become satiating over time (of circa 5–15 minutes).

In addition, it is known that the flavours/odours of foods are associated with the post-ingestive consequences of those foods.[153-156] Each food odour is associated with foods that

differ in their satiating capacities. These associations affect the hedonic value that is attributed to odours,[157] and guide food choice and food intake.[153, 158] Therefore, different odour types may evoke different degrees of appetite and/or satiety responses.

So far, it is unclear whether odour exposure time and/or odour type determine the appetizing or satiating responses to odours. The objective of the present study was to investigate the effect of odour exposure on general appetite and SSA over time.

Materials and methods

Experimental design

A cross-over design with eight different odour types (five food odours, two non-food odours and no-odour) was used. The experiment took place between 10.45 and 13.30 h. Each participant was scheduled on six days, preferably once per week at the same time of the day. All participants completed two sessions on one day, an early and a late session, with a break of 40 minutes in-between. In total, they completed 12 sessions, starting with a practice session to familiarize the participants with the procedure. The remaining 11 sessions were randomized over the subjects and the test days. Three of the eight odour types (chocolate, meat and no-odour) were tested twice per person to check the repeatability: once during the early session and once during the late session of a test day. The other five odour types were tested once per person.

Participants

Twenty-one healthy women, aged 18–45 years and BMI 18.5–25 kg/m^2 were recruited from Wageningen and surroundings. Exclusion criteria were as follows: restrained eating (Dutch Eating Behavior Questionnaire score>2.9),[159] smoking, pregnancy or breast feeding during the last 6 months, lack of appetite, following an energy restricted diet or change in body weight>5 kg during the last 2 months, hypersensitivity to food products under study and being a vegetarian. The participants were told that they participated in pilot tests to investigate the natural variation in hunger and salivation. After the study, the participants were informed about the real objectives. All participants signed an informed consent form. The study was approved by the Medical Ethical Committee of Wageningen University.

Odours

Seven odours were used: two sweet odours (chocolate and banana), two savoury odours (meat/savoury and tomato soup), one staple food odour (baked bread) and two non-food odours (pine tree and fresh green/grassy). Table 2.1 shows the details of the odours and their preparation.

Table 2.1 Odour preparation and snack choice.

Odour	Ingredient 1	Ingredient 2	Method[1]	Refresh rate	Snack 1[4]	Snack 2[4]
Banana	5g banana flavour (97151123, Givaudan)	25g propylene glycol (Merck, Amsterdam, the Netherlands)	Vaporiser Comp1: int=3min/run=6s	30min	banana	bread roll with jam
Chocolate	10g chocolate flavour (97532067, Givaudan)	undiluted	Vaporiser Comp1: int=1min/run=60s	30min	bread roll with chocolate spread	bread roll with jam
Meat	3.5g 'savory' flavour (96900240, Givaudan)	7.5g propylene glycol (Merck, Amsterdam, the Netherlands)	Vaporiser Comp1: int=3min/run=6s Comp2: int=2min/run=10s	30min	bread roll with steamed meat	bread roll with savoury salad (egg or celery)
Tomato soup	2g tomato flavour (15.01.0166, IFF)	250g Unox tomato soup[2] (Unox, stevige tomaten crème soep, Netherlands)	Vaporiser Comp1: int=3min/run=6s	20min	175g tomato soup	bread roll with savoury salad (egg or celery)
Bread	Prebaked baguette (Euroshopper, AH, Netherlands)		200°C in oven	10min[3]	bread roll with jam	bread roll with savoury salad (egg or celery)
Pine tree	5g Kneipp bath oil	7.5g propylene glycol (Merck, Amsterdam, the Netherlands)	Vaporiser Comp1: int=3min/run=6s Comp2: int=2min/run=10s	30min	bread roll with jam	bread roll with savoury salad (egg or celery)
Fresh green	Full bottle odour solution (819, AllSens Geurbeleving)	-	bottle without lid in room (diffusion)	-	bread roll with jam	bread roll with savoury salad (egg or celery)
No-odour	-	-	-	-	bread roll with jam	bread roll with savoury salads (egg or celery)

[1] int = interval time, run = run time, ComP=compressor, Comp1 (AG1503), Comp2 (AG1501), Voitair aroma factory, Martinsreed, Germany

[2] The tomato soup was heated in a microwave for 90s at 600W and wrapped with aluminium foil

[3] Two baguettes were placed in the oven. Every 10min 1 of them was replaced

[4] Participants could choose between snack 1 and snack 2

All odours were distributed in four identical air-conditioned rooms (Mood rooms, Restaurant of the Future, Wageningen, The Netherlands). Prior to the actual experiment, groups of naive persons judged around 40 different odours on pleasantness and perceived intensity. Only odours that were considered as pleasant by at least 8 out of 10 persons (oral evaluation) were included. Subsequently, odour concentration was adjusted to maximize the pleasantness, while aiming at equal intensities.[160] The pine tree and grass odours were perceived as dominant at intensity levels equal to those of the food odours and were therefore lower in intensity. The bread odour was distributed by baking baguettes in a microwave oven that was placed out of sight from the participants. For the fresh green odour, an open jar with odour solution was placed out of sight. The remaining five odours were dispersed via vaporizers (AllSens Geurbeleving, Oosterhout, The Netherlands), filled with odour dilutions. A compressor led clean air through the head space of the odour dilution (Table 2.1).[40]

Measurements

Three measurements were taken during the experiment following the procedure hereunder.

The appetite questionnaire measured hunger, desire-to-eat and thirst over time on 100 mm computerized visual analogue scales (VAS, not at all – very).[21] Besides the 'general' appetite, the appetite for 15 individual foods was measured by using 100 mm VAS in a randomised order (for example, 'How large is your appetite for a brownie at this moment?; not at all – very).[21] These foods were divided into odour-specific foods and reference foods. Odour-specific foods were foods associated with the odour to which participants were exposed to during the experiment: for example, beef soup during exposure to meat odour and brownie during exposure to chocolate odour. Seven of the foods were odour-specific foods: bread roll with steamed meat (meat odour), beef soup (meat odour), bread roll with chocolate sprinkles (chocolate odour), brownie (chocolate odour), banana pie (banana odour), tomato soup (tomato soup odour) and plain bread roll (bread odour). Eight foods were reference foods and were not related to any of the odours in this experiment: mushroom soup (savoury), curry soup (savoury), bread roll with egg (savoury), bread roll with jam (sweet), sweet pastry 'tompouce' (sweet), apple pie (sweet), pancake without topping (staple), croissant (staple). Odour intensity (100 mm VAS, not at all – very) was included to the appetite questionnaire to check whether participants were able to perceive the odours consciously during the 20 minutes of odour exposure. Odour pleasantness was not measured, because attention to the hedonic value affects brain processing of olfactory stimuli.[161]

The food preference questionnaire was a computerized task[162, 163] (E-prime, v2.0; Psychology software tools, Sharpsburg, PA, USA) measuring food preference after the 20 minutes of odour exposure. On each trial, the participant had to choose between two foods that were shown simultaneously on a computer screen by means of digital colour photographs. The foods on the photographs were the same as the foods in the appetite questionnaire, plus additional photographs of banana sweets, chocolate M&M's, cherry tomatoes and sausages. The frequency with which each food was chosen was determined.

Salivation was measured by placing a dental cotton roll (Salivette; 51.1534, Sarstedt, Nümbrecht, Germany) for 30 s under the tongue.[37, 82]

Standardizing hunger state

The visits of each participant were scheduled at the same time of the day to standardize the individual hunger state. On the first test day, participants were instructed to consume a normal amount of breakfast until 2.5 h before the start of the experiment. After this time, only water and weak tea were allowed. Participants were requested to drink 0.5 L of water 1 h before the start of the experiment to prevent possible dehydration. On the remaining five test days, the participants were requested to consume the same breakfast as on the first test day.

Procedure

The 'early' session started with baseline measurements (t=0) in a room with no-odour. Participants filled out the appetite questionnaire and collected saliva. Subsequently, each participant entered one of the test rooms that contained either one of the odours or no-odour. The participants were given instructions on a computer (E-prime, v2.0) to repeat the appetite questionnaire 1, 5, 9, 13 and 18 minutes and to collect saliva 0.5, 4, 8 and 17 minutes after entering the room. After 20 minutes, the participants entered another room with no-odour to complete the food preference questionnaire. When finished, participants received a snack (Table 2.1) to reinforce the association between the food odours and food intake[164, 165] and to compensate for the increase in hunger between the early and late session. The snacks contained 20% of a standard lunch, which is around 4% of the daily intake.[166] The daily intake was based on the average energy requirement for a woman in the study population (Scholfield I equation).[167] After a 40-minute break, the 'late' session of the test day started, and the whole procedure was repeated with another odour type. At the end of the study, participants were asked about their thoughts on the study objective in the end evaluation

Data analysis

Statistical analyses were performed with SAS (version 9.1.3; SAS Institute Inc., Cary, NC). Results are presented as mean values ± SD. P-values<0.05 (two-sided) were considered significant.

Rated hunger and desire-to-eat scores from the appetite questionnaire were averaged to analyse the effect of odour exposure on general appetite. The development SSA was determined with two methods. First, SSA was measured with the appetite questionnaire on VAS during odour exposure by asking 15 questions about the appetite for 15 separate foods. SSA was calculated as the average change in appetite for odour-specific foods minus the average change in appetite for reference foods. The change in appetite was calculated by subtracting the ratings before odour exposure from the ratings after exposure. SSA developed when SSA >0, while SSS developed when SSA <0. Secondly, development of SSA was determined with the food preference questionnaire, 20 minutes after the onset of odour exposure. The choice frequency was the number of times that a food was chosen. The 'delta choice' was calculated per food as the choice frequency of that food in the odour condition minus the choice frequency of that food in the no-odour condition. SSA developed when delta choice of the odour-specific food >0.

Differences between odour types (five food odours, two non-food odours and no-odour) were investigated with linear mixed models, variance matrix 'compound symmetry', and 'participant' in the repeated statement. The dependent variables were odour intensity, general appetite, SSA, appetite for sweet foods, appetite for savoury foods, appetite for staple foods, delta choice and salivation. The data measured by using VAS (intensity, general appetite, SSA and appetite for sweet/savoury/staple foods) and salivation were analysed with 'odour type' and 'exposure time' as fixed factors, including an 'odour type*exposure time' interaction. 'Time of the experiment' was included as co-variable to take a possible difference between the early and late session into account, when analysing data on general appetite, SSA, and appetite for sweet/savoury/staple foods. The VAS ratings at baseline (t=0) were included as co-variable for analysis on general appetite. The data collected with the food preference questionnaire (delta choice) were analysed with 'odour type' as fixed factor and 'time of the experiment' as co-variable.

To investigate the effect of food odours and non-food odours on general appetite, the results of all odour types were split into three categories: food odours, non-food odours and no-odour. Furthermore, the results of the food odours were split into sweet (banana and chocolate), savoury (tomato soup and meat) and bread odours to investigate the effect of odour type on general appetite and SSA in more detail. All outcomes were

checked for possible differences between the early and late session (data not shown). Data from nine sessions were missing due to scheduling difficulties and odour contamination. One participant joined an extra chocolate odour session instead.

Results

Odour intensity

During odour exposure, the odour intensity in each odour condition was rated as higher than in the no-odour condition, on every time point measured (all P<0.01; Figure 2.1). On average, intensity decreased by 16 mm on a 100 mm VAS in 18 minutes (P<0.001), which indicates there was some adaptation over time.

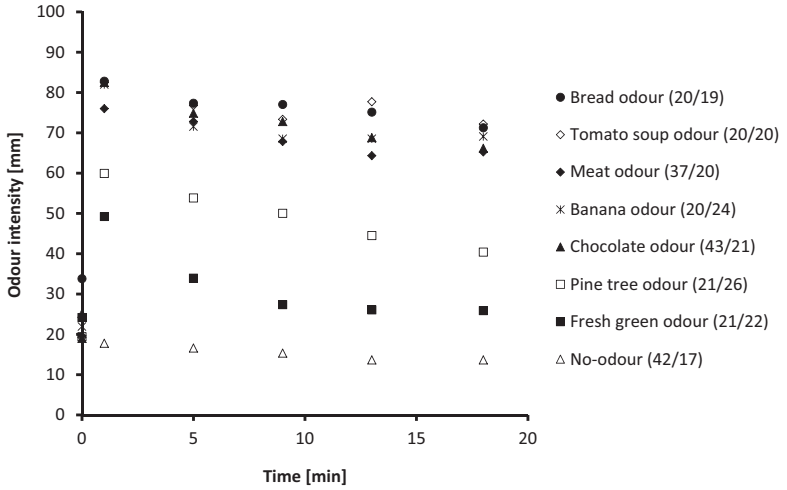


Figure 2.1 Mean odour intensity in all test conditions over time, measured by using 100 mm VAS. The numbers between the brackets represent (number of observations/average SD).

General appetite

Over all conditions, rated general appetite increased over time ($F_{4,80}$=9.09; P<0.001; Figure 2.2). Furthermore, general appetite differed significantly across odour types ($F_{7, 136}$=6.50; P<0.001). The interaction between odour type and exposure time was not significant ($F_{28,541}$=0.34; P=0.99), meaning that the effect of odour type on general appetite did not change over time. *Post-hoc* comparisons showed that food odours increased general appetite (P=0.010) while non-food odours suppressed general appetite (P=0.011), compared with no-odour. General appetite was greater after exposure to food odours than after non-food odours (P<0.001). Splitting the food odours into savoury, sweet and bread showed that both savoury (P=0.0037) and sweet odours (P=0.048) increased general appetite whereas bread odours did not (P=0.30), compared with no-odour.

The difference in general appetite between sweet and savoury odours was not significant (P=0.26).

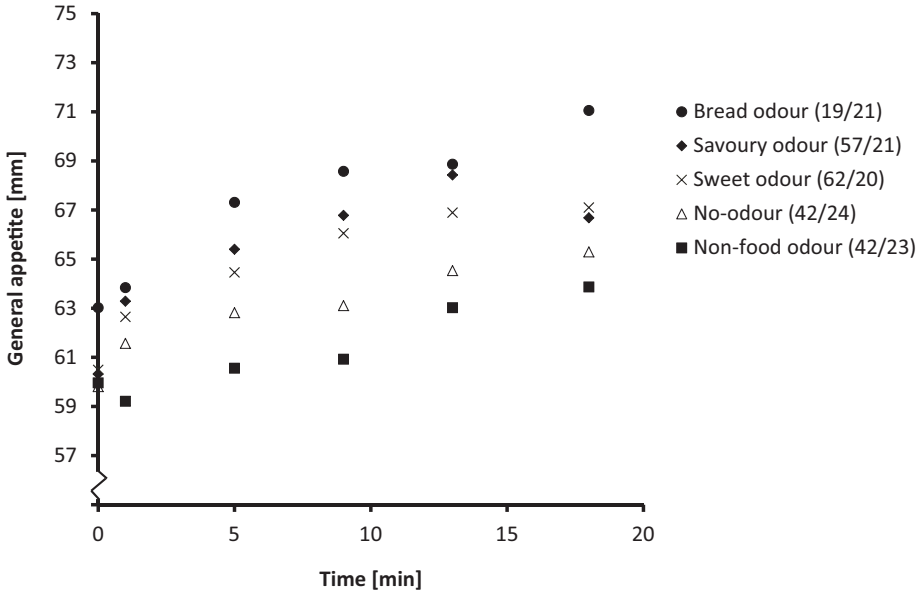


Figure 2.2 Mean appetite during exposure to savoury odours, sweet odours, bread odour, non-food odours and no-odour, measured by using 100 mm VAS. The numbers between the brackets represent (number of observations/average SD).

Sensory-specific appetite (SSA)

Each food odour stimulated the appetite for the odour-specific foods (average increase 12 ± 24 mm), relative to the change in appetite for reference foods (on average 1 ± 26 mm; P<0.001), which means by definition that sensory-specific appetite (SSA) had developed (Figure 2.3). SSA was affected by food odour type ($F_{4,76}$=17.86; P<0.001), but did not change over time ($F_{4,80}$=0.31; P=0.87). The interaction between food odour type and exposure time was not significant ($F_{16,304}$=0.33; P=0.99). *Post-hoc* comparisons revealed that SSA was larger after smelling savoury, than after smelling sweet (P<0.001). All food odours individually evoked SSA (all P<0.001). Non-food odours did not significantly affect the appetite for the reference foods (P=0.71).

The food preference questionnaire data confirmed the development of SSA for each food odour after 20 minutes of odour exposure (Figure 2.4A). Compared with the no-odour condition, the choice for banana products specifically increased after exposure to banana odour (P<0.001). Similarly, the choice for chocolate, meat, and tomato soup increased after exposure to respectively chocolate, meat and tomato soup odours (all P<0.001), compared with no-odour.

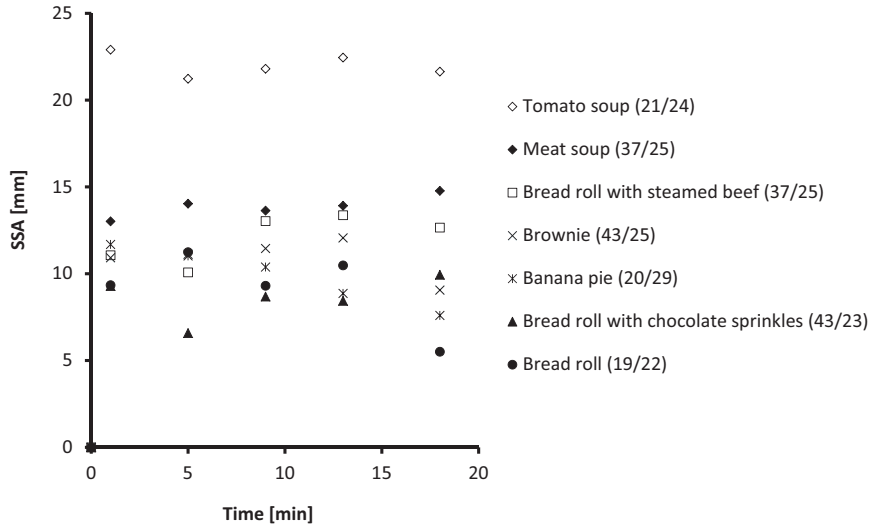


Figure 2.3 Calculated SSA per odour-specific food over time. SSA was calculated as the change in appetite for odour-specific foods minus the average change in appetite for reference foods from ratings on 100 mm VAS. For example, the change in appetite for tomato soup minus the average change in appetite for reference foods, during exposure to tomato soup odour. The numbers between the brackets represent (number of observations/average SD).

Category-specific response to food odours

The effect of food odours on the appetite for entire food categories (sweet, savoury and staple) was calculated from the food preference questionnaire (Figure 2.4B) and the appetite questions (VAS; Figure 2.5). Figure 2.4B shows that chocolate and banana odour increased the choice for sweet foods, but decreased the choice for savoury foods, compared with no-odour (both $P<0.01$). Meat and tomato soup odour showed the opposite effect (both $P<0.01$). Non-food odours and bread odour had no effect on the choice for sweet or savoury foods ($P>>0.05$).

Figure 2.5 shows the average change in appetite for odour-specific and category-specific foods during odour exposure, measured by using VAS. The data from the meat odour and the chocolate odour follow a similar pattern as the data from respectively the tomato soup odour and the banana odour (Table 2.2). The VAS data show that the tomato soup and meat odours increased the appetite for savoury foods ($P<0.001$), but decreased the appetite for sweet foods ($P<0.001$). Similarly, the banana and chocolate odours increased the appetite for sweet foods ($P=0.005$), but decreased the appetite for savoury foods ($P=0.002$). Sweet and savoury odours had no effect on the appetite for staple foods (both $P>0.05$). Bread odour increased the appetite for staple ($P<0.001$) and sweet foods ($P=0.006$), but did not significantly affect the appetite for savoury foods ($P=0.37$). The non-food odours had no effect on the appetite for sweet or savoury foods (all $P>0.05$).

Furthermore, the effect of food odours on the appetite for odour-specific foods (12 ± 24 mm) was larger than the effect on the appetite for category-specific foods (4 ± 11 mm; P<0.001).

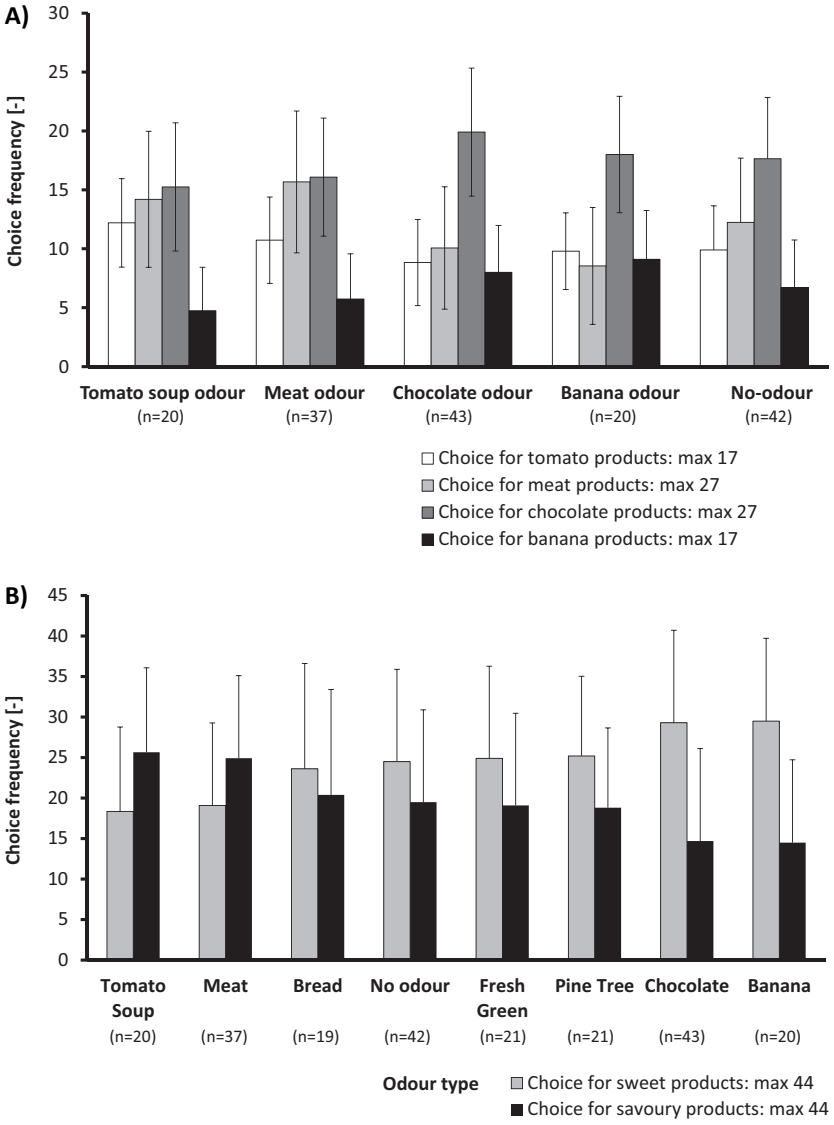


Figure 2.4 (A) Choice frequency of banana, chocolate, meat and tomato foods after exposure to different odours, measured with the food preference questionnaire. 'Max' represents the maximum choice frequency of a food. **(B)** Choice frequency of sweet and savoury foods after exposure to different odours, measured with the food preference questionnaire. 'Max' represents the maximum choice frequency of a food.

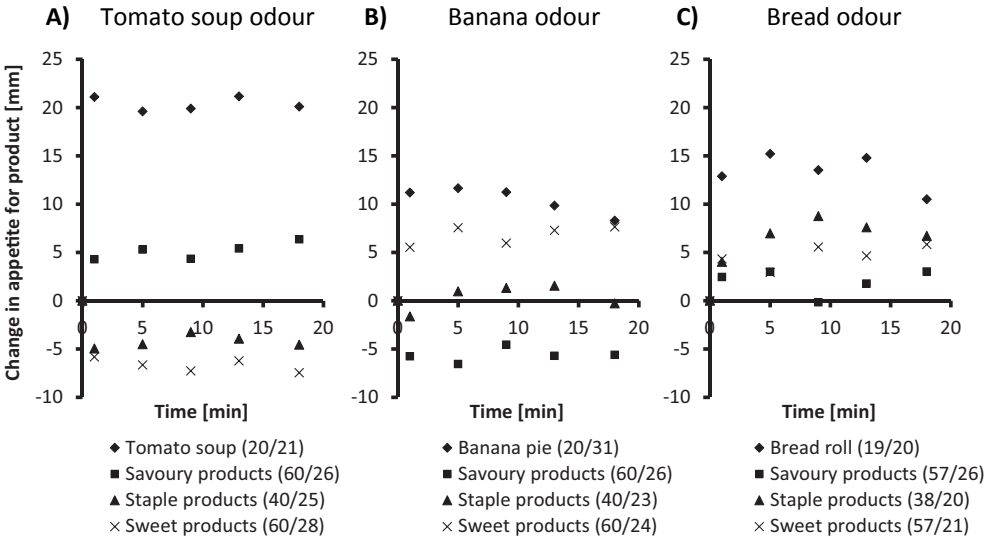


Figure 2.5 Mean change in appetite for odour-specific and category-specific foods, during exposure to **(A)** tomato soup odour, **(B)** banana odour and **(C)** bread odour, measured by using 100 mm VAS. The numbers between the brackets represent (number of observations/average SD).

Table 2.2 Mean change in appetite ± SD for separate foods, averaged over all times, during exposure to different kinds of foods, measured by using 100 mm VAS.

	Odour							
Product	No-odour	Tomato soup	Meat	Banana	Chocolate	Bread	Pine tree	Fresh green
Tomato soup	2 ± 29	**20 ± 21**	5 ± 23	-8 ± 28	-4 ± 29	1 ± 28	5 ± 28	2 ± 28
Mushroom soup*	0 ± 30	6 ± 27	9 ± 26	-7 ± 27	-5 ± 28	1 ± 29	5 ± 30	1 ± 30
Meat soup	1 ± 27	6 ± 24	**15 ± 24**	-6 ± 23	-4 ± 25	0 ± 26	5 ± 26	-1 ± 23
Curry soup*	0 ± 27	5 ± 26	6 ± 25	-5 ± 28	-2 ± 26	3 ± 24	6 ± 26	-3 ± 25
Bread roll with steamed meat	3 ± 27	6 ± 25	**13 ± 25**	-7 ± 23	-5 ± 25	4 ± 29	2 ± 25	-3 ± 26
Bread roll with egg*	0 ± 26	5 ± 24	2 ± 20	-5 ± 22	-4 ± 25	2 ± 25	3 ± 23	-3 ± 24
Bread roll with chocolate sprinkles	1 ± 22	-8 ± 24	-3 ± 20	3 ± 21	**9 ± 19**	3 ± 22	-1 ± 22	-1 ± 22
Bread roll with jam*	-1 ± 22	-3 ± 21	-4 ± 20	4 ± 22	0 ± 21	5 ± 21	3 ± 23	0 ± 21
Brownie	3 ± 27	-8 ± 33	-2 ± 27	6 ± 24	**11 ± 22**	7 ± 22	-2 ± 29	1 ± 27
Apple pie*	4 ± 25	-8 ± 30	-3 ± 26	7 ± 26	2 ± 24	7 ± 19	-2 ± 27	3 ± 25
Banana pie	0 ± 27	-5 ± 29	-4 ± 27	**10 ± 31**	5 ± 26	3 ± 25	2 ± 30	2 ± 26
Dutch sweet pastry 'tompouce'*	1 ± 29	-8 ± 31	-4 ± 27	10 ± 23	4 ± 24	1 ± 23	-2 ± 29	1 ± 25
Bread roll	-1 ± 21	-1 ± 21	2 ± 19	-2 ± 19	-2 ± 20	**13 ± 20**	-1 ± 21	-1 ± 18
Pancake*	3 ± 23	-6 ± 26	-2 ± 21	2 ± 22	2 ± 23	6 ± 19	2 ± 26	3 ± 23
Croissant*	3 ± 23	-3 ± 25	1 ± 24	-2 ± 23	2 ± 23	7 ± 20	2 ± 26	5 ± 25

* Reference food

Salivation

Saliva production was on average 0.27 ± 0.22g. No differences were found in salivation between odour types ($F_{7,136}$=1.89, P=0.075) or exposure time ($F_{4,80}$=1.53; P=0.20). The interaction between odour type and exposure time was also not significant ($F_{28,541}$=0.92; P=0.58).

End evaluation

None of the participants correctly guessed the complete purpose of the study, although all participants suspected that odours were involved. Seven thought we investigated the effect of odours on hunger. Nineteen participants reported becoming hungry from the food odours, and one person said the odours did not make her hungry, but changed her food preference.

Discussion

The objective of the study was to investigate the effect of odour exposure on general appetite and SSA over time. SSA is defined as an increase in the appetite for odour-specific foods relative to the appetite for reference foods. Remarkably, all food odours increased SSA up to 20 minutes exposure (Figure 2.3), while we expected a decrease in SSA (corresponding with the development of SSS) within five to ten minutes, based on other studies.[44, 57] In addition, food odours also increased general appetite irrespective of time (Figure 2.2). We suppose that exposure to food odours notified participants about possible food availability. Possibly, anticipation of food intake and the corresponding cephalic phase responses caused the increase in general appetite and SSA.[35, 42] Furthermore, the food odours had a relatively large influence on SSA and a small influence on general appetite. Apparently, the appetizing role of odours lays mainly in directing food choice and is not dependent on exposure time.

Several factors may be important for the development of SSS, which is the opposite of SSA: nutrient intake, route of odour perception, oral stimulation, perceived intensity and attention. Originally, SSS has been explained by the need for variety in our meal to gain a balanced diet.[11] When foods are merely smelled, like in the present study, no nutrients are ingested. Therefore, the consumption of the cued food remains desirable. However, experiments with modified sham feeding and chewing gum showed that SSS developed even without nutrient intake.[57, 141-143, 168, 169] Possibly, these artificial ways of exposure to sensory signals deceive our body or the retronasal odours (perceived via the mouth) have a role. It has been suggested that retronasal odours are related to reward, whereas orthonasal odours (perceived via the nose like in the present study) are related to

anticipation.[124, 125] Possibly, retronasal odours are linked to nutrient intake and become satiating, whereas orthonasal odours are appetizing. In addition, oral exposure may also affect SSS, because cephalic phase responses are generally stronger during oral than during odour stimulation.[149, 170] Finally, Rolls and Rolls,[57] and Jansen *et al.*[44] showed development of SSS even though no nutrients were ingested and odours were presented orthonasally. They gave instructions to smell intensely, which may have drawn attention to the odours and increased the perceived intensity, consequently affecting SSS. In contrast to literature,[44, 57] we found no indications that orthonasal food odours become satiating over time. In connection with our results, a recent study found that food intake also was not affected by odour exposure time.[45]

Besides increasing the appetite for odour-specific foods, food odours influenced the appetite for other foods (Table 2.2). The savoury odours increased the appetite for all savoury foods and decreased appetite for all sweet foods, whereas the opposite was found for the sweet odours. Exposure to bread odour increased the appetite for all foods, whereas non-food odours had no effect on the appetite for the reference foods. Other researchers also observed a generalization across foods within the same sweet/savoury category after exposure to the sight and smell of foods.[37, 38] Moreover, category-specific encoding in olfaction was found for food odours and non-food odours.[171, 172] Apparently, odours can be categorized into sweet, savoury, perhaps staple and non-food odours.

This categorization may be due to the association of each odour to foods with a certain macronutrient composition. It has been suggested that savoury foods are associated with protein-rich foods and sweet foods with sugars.[28, 31] Each macronutrient is digested in a specific way. It would be plausible if also the magnitude of the cephalic phase responses depend on macronutrient composition, as suggested by Smeets *et al.*[43] Macronutrients may, for example, influence saliva composition.[173] Such food-specific cephalic phase responses may also be induced by their associated odours. The increase in appetite for congruent foods after food odour exposure is consistent with the theory that food cues prepare the body for intake of the cued food by means of cephalic phase responses.[42, 48] The decrease in appetite for incongruent foods after food odour exposure might suggest that, once the body is prepared for intake of a certain food with a particular macronutrient composition, it is less favourable to consume foods that are very different from the cued food. Therefore, we propose that odours can evoke odour-specific cephalic phase responses.

In addition, the effect of food odours on the appetite for odour-specific foods was more than twice as large as the effect on the appetite for category-specific foods (Figure 2.5).

Probably, the more similar the odour and the food were, the larger was SSA. This finding parallels with the development of SSS after food intake: SSS transfers to uneaten foods that have similar properties as the eaten food. The more similar uneaten food to eaten food, the larger the SSS of the uneaten food.[11, 13, 58, 174]

In the present study, food odours increased general appetite whereas non-food odours decreased general appetite, compared with the no-odour condition (Figure 2.2). The effect persisted over time even though there was a slight adaptation to the odours. Moreover, general appetite increased over time in all conditions, even in the no-odour condition. Besides a natural increase in general appetite during lunchtime, general appetite may have increased during the experiment by answering the questions about appetite for several foods, because even thoughts of foods can induce cephalic phase responses and increase appetite.[34, 170]

Finally, we did not find an effect of odour exposure on salivation whereas other studies repeatedly did.[149, 171] Ferriday *et al.*[37] reported an increase in salivation after exposure to sight and smell of pizza using the same method, but only in overweight participants. We believe that 30 s was perhaps not long enough to collect enough saliva in normal-weight people to detect any differences.

Several factors may have influenced the outcomes of the study, such as the time of the experiment (lunchtime). However, we did not observe any differences in the outcomes between the early and the late session of the day (data not shown). Furthermore, only pleasant odours were selected to minimize possible effects of odour pleasantness on the appetizing responses to the odours.[175] Finally, the magnitude of the SSA probably depends on the similarity between the chosen odours and odour-specific foods. The tomato soup odour and the tomato soup were very similar, because real tomato soup was used to distribute the odour. The banana odour, on the other hand, smelled like banana sweets and the resemblance with the banana pie (odour-specific food) was less strong.

In conclusion, palatable food odours were appetizing during and after odour exposure and did not become satiating over time. Food odours had a large impact on SSA and a small impact on general appetite. Furthermore, exposure to food odours increased the appetite for congruent foods, which is consistent with the theory that food cues prepare the body for food intake. Interestingly, exposure to food odours also decreased the appetite for incongruent foods, implicating a disadvantage of consuming foods that are very different from the cued food. It may be hypothesised that once the body is prepared for intake of a certain food with a particular macronutrient composition, it is unfavourable to consume

foods that are very different from the cued food. Further research could investigate whether food odours affect actual food choice.

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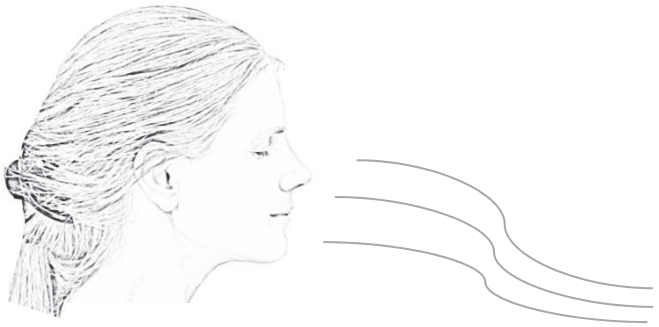
Conflict of interest

The authors declare no conflict of interest.

Chapter 2B

Orthonasal passive odour exposure



A pilot study to explore if food preference depends on odour exposure time

Mariëlle Ramaekers, Sanne Boesveldt, Catriona Lakemond, Martinus van Boekel, Pieternel Luning

Abstract

Background: Food preference may change over time response to food odours. Possibly, a 1 minute exposure to food odours stimulates the preference for foods that were smelled, while a twenty minute exposure suppresses the preference for smelled foods.

Objective: A pilot study to explore if food preference changes over time during odour exposure.

Design: In a 3x2 within-subject design, 13 unrestrained women (age: 18-45 y; BMI: 18.5-25 kg/m^2) were exposed for one or twenty minutes to meat odour, chocolate odour and no-odour in six separate sessions. All odours were distributed in a test room at supra-threshold levels. Sensory-specific appetite and general appetite were measured by using visual analogue scales, during odour exposure. After the odour exposure, food preference was assessed with a computerised task presenting 132 trials of two food pictures.

Results: The exposure time, i.e. 1 or 20 minutes, did not affect food preference. Furthermore, participants more often chose chocolate and sweet products after exposure to the chocolate odour, and less often meat and savoury products compared with the no-odour conditions. However, the preferences in the meat odour condition and the no-odour condition were similar. The sensory-specific appetite ratings on VAS revealed an increase in the appetite for the smelled food in both the meat and the chocolate odour condition.

Conclusions: Food odours appear to prime people to choose what they smelled, independently of the exposure time to the food odours.

Keywords: Appetite, Food Preference, Odour type, Olfactory cue, Sensory-specific appetite, Sensory-specific satiation

Introduction

Nowadays, many people have unhealthy eating habits with frequent ingestion of high caloric foods and drinks that lack nutritional value.[3] In this obesogenic society, food intake and food preferences are steered by a number of factors[3] under which food cues play a role.[33-37]

Although much research underscores the appetizing effects of food cues,[33, 36, 38, 48, 61, 140, 148, 149] there are several studies that found a (sensory-specific) satiating response to a longer exposure time to food cues.[44, 57, 121, 143, 152] Sensory-specific satiation (SSS) is the decrease in the pleasantness or desire-to-eat (DTE) of eaten foods relative to uneaten foods.[11, 12] Modified sham feeding (tasting but not swallowing) decreased the pleasantness of the food.[57, 141-143] Decreases in food intake or appetite might be explained by sensory-specific satiety, It is thought that also exposure to food cues, such as odours,[57] or pictures[176] may induce SSS. Rolls and Rolls[57] found a decrease in odour pleasantness after smelling banana for five minutes. Larson *et al.*[176] showed that the enjoyment (=reward) of eating peanuts was lower after evaluating how appetizing pictures with savoury food were, whereas rating pictures with sweet foods did not affect enjoyment of eating peanuts. The studies that report a satiating response to food cues all expose their participants for at least five minutes to the food cue,[57, 121, 141-143] whereas all studies with a brief exposure to food cues report an appetizing response,[37, 38, 140] leading to the suggestion that exposure time is crucial for the differences in appetizing or satiating response to food cues.

In the present pilot study, the effects of a short (1 min) and a long (20 min) exposure to a sweet and a savoury odour on food preference were explored. We hypothesised that a 1 minute exposure to food odours stimulates the preference for foods that were smelled, while a twenty minute exposure suppresses the preference for smelled foods. The study aimed at getting a first insight into possible differences in food preference between long and short-term exposure and a limited statistical power was accepted prior to the start of the experiment.

Materials and methods

Experimental design

A 2x3 within-subject design was used for this pilot study, in which all participants were exposed to six odour conditions differing in exposure time (one or twenty minutes) and odour type (meat, chocolate and no-odour). The experiment took place between 10.45 h

and 13.30 h at the same days, time and in the same rooms as study 2A. Each participant was scheduled on seven days, preferably once per week at the same time of the day, with the first test day being a training session.

Participants

Thirteen healthy, normal weight women, aged 18–45 years and BMI 18.5-25 kg/m2 were recruited from Wageningen and surroundings. Exclusion criteria were: restrained eating (Dutch Eating Behavior Questionnaire score >2.9),[159] smoking, pregnancy or breast feeding during the last six months, lack of appetite, following an energy-restricted diet or change in body weight >5 kg during the last 2 months, hypersensitivity to food products under study, and being a vegetarian. The participants were told that they participated in pilot tests to investigate the natural variation in hunger and salivation. After the study, the participants were informed about the real objectives. All participants signed an informed consent form.

Odours

The same chocolate and meat odours were used as in study 2A. Table 2.1 shows the details of the odours and their preparation. Both odours were dispersed via vaporizers (AllSens Geurbeleving, Oosterhout, The Netherlands), in four identical air-conditioned rooms (Mood rooms, Restaurant of the Future, Wageningen, The Netherlands).

Measurements

Three measurements were taken during the experiment following the procedure below.

The food preference questionnaire was a computerized task[162, 163] (E-prime, v2.0; Psychology software tools, Sharpsburg, PA, USA) measuring food preference. On each trial, the participant had to choose between two foods that were shown simultaneously on a computer screen by means of digital colour photographs. The foods on the photographs were bread roll with chocolate sprinkles (chocolate), chocolate custard (chocolate), brownie (chocolate), bread roll jam (sweet), vanilla custard (sweet), banana pie (sweet) bread roll with steamed meat (meat), beef soup (meat), little snack sausages (meat), bread roll with egg (savoury), tomato soup (savoury) and cherry tomatoes (savoury). All products were compared twice against all other products, resulting in 132 comparisons. The frequency with which each food was chosen was determined.

The appetite questionnaire measured hunger, desire-to-eat and thirst over time on 100 mm computerized visual analogue scales (VAS, not at all – very).[21] Besides the 'general' appetite, the appetite for 15 individual foods was measured by using 100 mm VAS in a randomised order (for example, 'How large is your appetite for a brownie at this

moment?; not at all – very).[21] These foods were divided into odour-specific foods and reference foods. Odour-specific foods were foods associated with the odour to which participants were exposed to during the experiment: for example, beef soup during exposure to meat odour and brownie during exposure to chocolate odour. Four of the foods were odour-specific foods: bread roll with chocolate sprinkles (chocolate odour), brownie (chocolate odour), bread roll with steamed meat (meat odour) and beef soup (meat odour). Eleven foods were reference foods and were not related to any of the odours in this experiment: banana pie (sweet), bread roll with jam (sweet), sweet pastry 'tompouce' (sweet), apple pie (sweet), tomato soup (savoury), mushroom soup (savoury), curry soup (savoury), bread roll with egg (savoury), pancake without topping (staple), croissant (staple) and plain bread roll (staple). Odour intensity (100 mm VAS, not at all – very) was included to the appetite questionnaire to check whether participants were able to perceive the odours consciously during the 20 minutes of odour exposure. Odour pleasantness was not measured, because attention to the hedonic value affects brain processing of olfactory stimuli.[161]

Salivation was measured by placing a dental cotton roll (Salivette; 51.1534, Sarstedt, Nümbrecht, Germany) for 30s under the tongue.[10, 35]

Standardizing hunger state

The visits of each participant were scheduled at the same time of the day to standardize the individual hunger state. Exactly the same instructions were given for standardizing the hunger state as in study 2A. The participants were requested to consume a normal breakfast on the first test day, at least 2.5 h before the start of the experiment. After that, only weak tea and water were allowed with the obligation to drink 0.5 L of water one hour before the start of the experiment. On the remaining six test days, the participants were requested to consume the same breakfast as on the first test day.

Procedure

There were two types of conditions: a 'short' condition with an odour exposure time of one minute and a 'long' condition with an odour exposure time of twenty minutes. All sessions started with baseline measurements (t=0) in a room with no odour. Participants filled out the appetite questionnaire and collected saliva. In the 'short' conditions, the participants started with waiting for 17 minutes in the waiting room, while participants in the 'long' condition directly continued the test. Then the participants entered one of the test rooms that contained either chocolate, meat or no-odour. In the 'short' condition, the participants were given instructions on a computer (E-prime, v2.0) to collect saliva 0.5 minute after entering the room and to fill out the appetite questionnaire 1 minute and

after entering the room. In the 'long' condition, the participants were given instructions on a computer (E-prime, v2.0) to fill out the appetite questionnaire at 1 and 18 minutes and to collect saliva 0.5 and 17 minutes after entering the room. After that, the participants completed the food preference questionnaire in a different room with no odour. When finished, participants received a snack (Table 2.1) to reinforce the association between the food odours and food intake. [164, 165]

Data analysis

Statistical analyses were performed with SAS (version 9.1.3; SAS Institute Inc., Cary, NC). Results are presented as mean values ± SD. P-values <0.05 (two-sided) were considered significant. We removed the data from one session (meat-long) due to odour contamination.

The FPQ data were transformed using arcsine(sqrt(frequency/max)), with max representing the maximum number of times a product could be chosen in a set. All comparisons between products in the FPQ were split into six sets, with each set containing comparisons of products. For example, the comparison between brownie and beef soup belongs to the set 'choc-meat' and the comparison between banana pie and tomato soup belongs to set 6 'sweet-savoury' (Table 2.3).

Rated hunger and desire-to-eat scores from the appetite questionnaire were averaged to analyse the effect of odour exposure on general appetite. The development of sensory-specific appetite (SSA) was determined with the appetite questionnaire on VAS during odour exposure by asking 15 questions about the appetite for 15 separate foods. SSA was calculated as the average change in appetite for odour-specific foods minus the average change in appetite for reference foods. The change in appetite was calculated by subtracting the ratings before odour exposure from the ratings after exposure. SSA developed when SSA >0.

Differences between exposure times (1 minute 'short' and 20 minutes 'long') and odours (chocolate, meat and no-odour) were investigated with linear mixed models, with participant as factor with random effects. The FPQ data, with the arcsinus of the square root of the frequency as the dependent variable, were analysed with odour, exposure time, set and all their interactions as factors with fixed effects. General appetite and SSA were analysed with odour, exposure time and their interaction as factors with fixed effects. VAS ratings at baseline (t=0) were included as co-variable for analysis of general appetite. An unstructured covariance matrix specified the correlations between the preferences for banana, meat, sweet, savoury and staple products in the FPQ data. Degrees of freedom were calculated according to the method by Kenward and Roger.[177]

Results

Food preference

Exposure time, short vs long, had no significant effect on the preference for meat products (in sets 1, 4 and 5; $F_{1,59}$=0.2; P=0.65) or the preference for chocolate products (in sets 1,2 and 3; $F_{1,59}$=0.0; P=0.89; Table 2.3). Therefore, the data were subsequently grouped per odour. The chocolate odour increased the choice for the chocolate products (P<0.001) and decreased the choice for the meat products (P=0.001), compared with no-odour. The meat odour had no effect on the choice for chocolate (P=0.61) or meat products (P=0.65), compared with no-odour. Additionally, preferences were analysed per set, showing significant differences in set 1, chocolate vs meat, but not in the other sets (Table 2.3).

Table 2.3 Average percentage ± SD of times a product was chosen per set of products after exposure to different conditions, measured with the food preference questionnaire.

	Set					
	1	2	3	4	5	6
Product 1	**Choc**-	**Choc**-	**Choc**-	**Meat**-	**Meat**-	**Sweet**-
Product 2	Meat*	Sweet*	Savoury*	Sweet#	Savoury#	Savoury#
Chocolate - short	68 ± 27 a†	68 ± 18 †	58 ± 22 a	46 ± 35	45 ± 22	42 ± 31
Chocolate - long	71 ± 29	71 ± 21	59 ± 31	41 ± 30	37 ± 24	44 ± 29
No odour - short	57 ± 31 ‡	67 ± 15	45 ± 21 b	53 ± 30	41 ± 24	37 ± 22
No odour - long	50 ± 28	61 ± 23	44 ± 21	56 ± 29	47 ± 30	39 ± 23
Meat - short	51 ± 34 b	62 ± 20 ‡	41 ± 22 b	53 ± 29	45 ± 28	36 ± 25
Meat - long	50 ± 35	61 ± 15	45 ± 26	58 ± 32	50 ± 31	31 ± 22

Different letters (a,b) denote significant differences at P<0.05.

Different symbols (†‡) denote P<0.10.

*Average percentage of times a chocolate product was chosen.

Average percentage of times a meat product was chosen.

18 comparisons per set.

Sensory-specific appetite (SSA)

SSA did not differ between short or long exposure ($F_{1,60.3}$=0.1; P=0.79). SSA developed during exposure to chocolate (9 ± 11 mm; P=0.017) and meat (11 ± 16 mm; P=0.004) odour, averaged over times 1 and 18 min.

Additionally, the relative change in appetite for meat products (appetite for meat products minus appetite for reference products) was higher during exposure to meat odour (11 ± 16 mm) than in the no-odour (0 ± 10 mm; P=0.001) or the chocolate

(-4 ± 9 mm; P<0.001) condition. The relative change in appetite for chocolate products (appetite for chocolate products minus appetite for reference products) was higher during exposure to chocolate odour (9 ± 11 mm) than in the no-odour (2 ± 5 mm; P=0.002) or the meat (-1 ± 8 mm; P<0.001) condition.

General appetite

Ratings after one-minute exposure were used for the short conditions and ratings after eighteen-minutes exposure for the long conditions. Mean general appetite ratings were 70 ± 25 mm in condition chocolate-short, 72 ± 24 mm in chocolate-long, 72 ± 24 mm in meat-short, 69 ± 28 mm in meat-long, 69 ± 24 mm in no odour-short and 67 ± 22 mm in no odour-long. General appetite did not differ between short and long exposure ($F_{1,58.8}$=0.6; P=0.43). Furthermore, there was no significant effect of odour ($F_{2,58.5}$=0.3; P=0.78) or the interaction between odour and 'short vs long' exposure time ($F_{2,58.6}$=0.5; P=0.58).

Discussion

The main objective of this explorative study was to investigate if food preference depends on odour exposure time, measured with a food preference questionnaire. The exposure time, i.e. 1 or 20 minutes, did not affect food preference. This finding contrasts our hypothesis, but is complementary with the results on the appetites for specific products gathered with visual analogue scales in study 2A. Both studies clearly show that food preference is altered by exposure to food odours, but does not depend on the exposure time. Larsen *et al.*[45] found that food intake was not affected by odour exposure time. Therefore, it may be suggested that food odours prime our eating behaviour independent of exposure time to those odours.

Furthermore, exposure to chocolate odour increased the preference for chocolate (sign.) and sweet products (not sign.), and decreased the preference for meat (sign.) and savoury products (not sign.), compared with no-odour in the food preference questionnaire (FPQ). The VAS data reveal the same pattern during chocolate odour with a clear increase in the appetite for chocolate products, relative to the reference foods, named sensory-specific appetite. These data are also in line with our findings in study 2A. The preferences in the meat odour conditions, however, did not differ much from the no-odour condition, suggesting no or little effect of meat odour (FPQ). On the other hand, the VAS data clearly showed an increase in the appetite for meat products during exposure to meat odour relative to no-odour, similar to results in study 2A. In conclusion, food odours appear to

prime people to choose what they smelled and this effect does not change over time during odour exposure.

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Chapter 3A

Orthonasal active odour exposure

Sensory-specific appetite is affected by actively smelled food odours and remains stable over time in normal-weight women.

The Journal of Nutrition, 2014; **144**(8). Mariëlle Ramaekers, Sanne Boesveldt, Gerrit Gort, Catriona Lakemond, Martinus van Boekel, Pieternel Luning

Abstract

Understanding overconsumption starts with knowledge of how separate factors influence our eating behaviour. Food cues such as food odours are known for their effect on general appetite and sensory-specific appetite (SSA). Active sniffing rather than passive exposure may induce satiation over time. The objective of this study was to investigate how actively sniffing banana odours affects general appetite, SSA, and subsequent food intake. In a crossover study, 61 women actively smelled cups containing natural banana, artificial banana odour or water in duplo (no-odour) for 10 min. Treatment order was randomly assigned as much as possible. General appetite and SSA were monitored by using 100 mm visual analogue scales during the 10 min of active sniffing, followed by *ad libitum* intake of banana milkshake. Results showed that SSA was consistently high (+12 mm) during actively sniffing natural or artificial banana odours, with no decrease in SSA over time. Sniffing both banana odours increased the appetite for banana (+11 mm) and other sweet products (+4 mm), whereas the appetite for savoury products decreased by 7 mm (all $P<0.01$) compared with no-odour. Actively sniffing banana odour did not significantly influence food intake ($P=0.68$) or general appetite scores ($P=0.06$). In conclusion, SSA scores during active sniffing were identical to the SSA found in a similar study that used passive smelling, suggesting that SSA is independent of the manner of sniffing and independent of exposure time. Moreover, sweet/savoury categorization may suggest that food odours communicate information about the nutrient composition of their associated foods. These data clearly show the appetizing effects of food odours.

Keywords: Food cue, Food intake, General appetite, Olfaction, Sensory-specific satiety.

Introduction

In the current obesogenic environment, in which people are often exposed to food cues and energy-dense palatable foods are abundantly available,[1, 178] it is essential to understand how separate factors influence our eating behaviour to understand problems such as overconsumption. It has been suggested that exposure to food cues, such as the sight, taste, and smell of foods, increases appetite and encourages eating, even in the absence of physiologic hunger.[9, 33-37] Food cues also specifically influence the appetite for the cued food,[36-38, 140, 179] termed sensory-specific appetite (SSA).[179] In contrast to general appetite, SSA specifically refers to the appetite for the cued food and is considered the opposite of sensory-specific satiety (SSS). SSS represents the decrease in reward derived from the eaten food relative to uneaten foods.[11, 12] The present study explores the appetizing/satiating capacity of food odours when intensely sniffed during a 10-min period.

Previous studies demonstrated that general appetite and SSA increase during a short 1- to 3-min exposure to food cues.[34, 35, 37, 140] However, a longer exposure to food cues, i.e. 5-20 min leads to varying results, with an increase[36, 179] or a decrease[44, 57, 121, 141-143, 152, 168, 169] in general appetite and/or SSA. A 10-min exposure to cookie or pizza odour increased hunger and craving for the cued food.[36] Similarly, exposure to sweet, savoury, or bread odours consistently increased the general appetite and SSA during a 20-min period.[179] In addition, a 30-min exposure to ambient citrus odour increased the choice for citrus during a subsequent buffet.[40] In contrast, Massolt *et al.*[121] found that participants experienced less appetite and felt more satiated after smelling dark chocolate for five min. Rolls and Rolls[57] found that the pleasantness of the smell of bananas decreased after five min of intensely smelling bananas, relative to other foods, and named this effect 'olfactory SSS'. Ten minutes of intensely smelling foods decreased food intake in normal-weight children but not in obese children[44] and in restrained eaters but not unrestrained eaters,[46] which was attributed to SSS.[44] SSS also developed during modified sham feeding (tasting without swallowing)[57, 141-143] and chewing gum.[168, 169] Furthermore, Smeets *et al.*[152] reported metabolic changes after modified sham feeding related to satiety, such as an increase in insulin and a decrease in plasma glycerol.

So far, it is unclear why results for general appetite and SSA differ after longer exposures. In both studies that reported an increase in general appetite or SSA, the participants stayed in an odourised room without specific instructions regarding the odour,[36, 179] whereas in the odour studies by Rolls and Rolls,[57] Massolt *et al.*[121] and Jansen *et al.*,[44] the foods were smelled actively, which probably led to intensity enhancement[109] and

attention toward the stimuli. Participants in the modified shamfeeding studies were also consciously aware of the food in their mouth. Attention can change the perception and evaluation of a stimulus.[161, 180] Furthermore, sniffing was shown to influence the neural encoding in the olfactory system[181, 182] and sniffs tend to be longer when an individual is hungry.[145] There is possibly a relation between the manner of sniffing and appetite. Active sniffing, rather than passive exposure, may induce the satiating effects of odours over time.

The primary objective of the current study was to investigate the effect of actively sniffed food odours on appetite, SSA, and food intake over time. We hypothesised that a short exposure to actively smelled food odours leads to an increase in appetite and SSA,[33-35, 37, 140] whereas a 10-min exposure decreases appetite and SSA.[44, 57, 121, 141-143, 152, 168, 169] *Ad libitum* intake was incorporated to investigate the reactivity to food odours at a behavioural level.

Materials and methods

Experimental design

In a within-subject study, all participants were exposed to five odour conditions. The data from two conditions were removed from the results because of possible odour contamination. Therefore, only information on the remaining three conditions (natural banana, artificial banana and no-odour) is given. The no-odour condition was measured in duplo. Each participant was scheduled preferably once per week and preferably at the same time of the day. During each session, participants were asked to actively smell the contents of a cup for 10 min. The participants then consumed an *ad libitum* amount of banana milkshake. The experiment took place between 10.30 and 14.15 h on 28 test days. On each test day, no-odour or banana odour (natural or artificial) was offered to prevent odour contamination in the test room between the different odours. Treatment order was randomly assigned as much as possible, but restricted availability of the participants on the test days led to a slight imbalance.

Participants

Initially, 63 healthy, normal-weight women aged 18–45 years with a BMI of 18.5–25 kg/m^2 were recruited from Wageningen and surroundings (the Netherlands). Sixty-one women completed the study. Two participants dropped out because of other obligations. A sample size calculation revealed that a minimum of 58 participants was required. Exclusion criteria were as follows: dislike for banana, banana pie (Dutch pastry), steamed meat, or meat soup (score <5 on a 9-point scale); smoking; pregnancy; breastfeeding

during the last six months; lack of appetite; following an energy-restricted diet; change in body weight >5 kg during the past 2 months; hypersensitivity to any of the food products under study; or being a vegetarian. The participants were told that the influence of sensory signals on food choice was being investigated. Afterward, participants were informed about all of the study objectives. All participants signed an informed consent form. The Medical Ethical Committee of Wageningen University approved the study.

Odours

In addition to natural banana odour, artificial banana odour was used for comparison with the results from the artificial odours in our previous study.[179] Cups were filled with 10 g of water in the no-odour conditions, with a tablespoon of medium-ripe mashed banana in the natural banana condition, and with 0.1 g banana flavour (97151123, Givaudan) plus 9.9 g propylene glycol (Merck) in the artificial banana condition. The cups were covered with a tissue to prevent visual cues. A plastic lid was placed over the tissue when participants were not actively sniffing to avoid odour contamination. The intensity of the artificial odour was matched with the intensity of the natural banana odour in pilot tests (data not shown).

Standardizing hunger state

All participants completed their individual test sessions at the same time of the day to standardize the individual state of hunger. In addition, they consumed the same normal amount of breakfast on each test day, at least 2.5 h before the experiment.

Procedure

After arrival at the test location, the participants first filled out the appetite questionnaire at baseline (t=0; Figure 3.1). Subsequently, each participant went to a test room where instructions were given on a computer screen (E-prime, version 2.0; Psychology Software Tools). Participants were requested to remove the plastic lid from the cup in front of them, while keeping the tissue on the cup, and intensely sniff the contents of the cup with their nose above the tissue. They were encouraged to continue intense smelling during the following 10 min. The participants repeated the appetite questionnaire 1, 5, and 9 min after entering the room. After 10 min, the participants went to a lunchroom where they consumed the *ad libitum* lunch for 15 min. The appetite questionnaire was repeated before and after lunch.

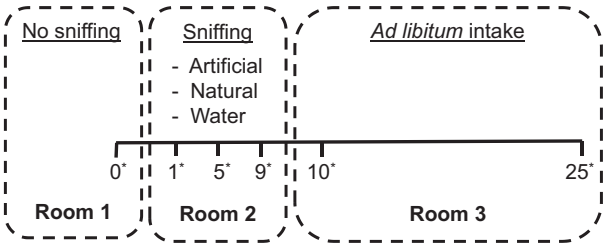

Figure 3.1 Schedule of a participant during a test session. *Timing (in min) of appetite ratings by using visual analogue scales.

Appetite questionnaire

An appetite questionnaire measured hunger ('How hungry are you at this moment?') and desire to eat ('How big is your appetite at this moment?') over time on 100 mm computerized visual analogue scales (VAS, not at all – very).[21] In addition to 'general' appetite, the appetite for 11 individual products was measured by using a 100 mm VAS in a randomly assigned order to determine SSA (e.g., 'How large is your appetite for a brownie at this moment?'; not at all – very).[21] These products were successfully used in a previous study[179] and were divided into banana, sweet, savoury and staple products. Banana pie and banana milkshake were selected as banana products. Sweet reference products were sweet pastry 'tompouce', strawberry milkshake, and brownie. Savoury reference products were mushroom soup, bread roll with egg, and tomato soup. Bread roll, pancake, and croissant were selected as staple reference products. In addition, the expected pleasantness of banana milkshake and meat soup were rated by using a 100 mm VAS without actually tasting these foods (e.g., 'How pleasant would you rate the taste of meat soup at this moment?'; not at all – very).[21] Finally, odour intensity ('How strong is the odour at this moment?'; 100 mm VAS, not at all – very) was added to the appetite questionnaire.

Ad libitum lunch

The participants ate lunch, which consisted of three compulsory small bread rolls (25 g each) with a chosen topping of strawberry jam (25 g, Geurts) or chocolate spread (15 g, Nutella; Ferrero). At the same time, the participants consumed an *ad libitum* amount of banana milkshake, consisting of 0.75 L banana drink (Maaza, Infra Foodbrands BV), 0.3 L semiskimmed milk (C1000) and 0.45 L full-fat yogurt (C1000) in a large beaker. The banana milkshake contained 22.1 g/kg protein, 81.3 g/kg carbohydrates, 12.0 g/kg fat, and 2192 kJ/kg energy. All bread rolls were consumed, and none of the participants finished the 1.5-L banana milkshake.

Data analysis

The effects of odour (i.e., natural, artificial and no-odour) and exposure time on general appetite (variable 1), SSA (variable 2), change in appetite for specific products (variable 3), change in expected pleasantness of specific products (variable 4), food intake (variable 5), and odour intensity (variable 6) were analysed by using mixed linear models.[183] Mixed models contain a fixed part consisting of the experimental factors and covariates and a random part to analyse the correlations between observations that were caused by the repeated measures per participant. For food intake, the fixed part consisted of experimental factor odour and the covariates general appetite at baseline (t=0), starting time, and starting time squared. Starting time was the time a participant started a session. For all other variables, the fixed part contained the main and interaction effects of odour and exposure time. In addition, general appetite at baseline (t=0) was included as a covariate for analysis of general appetite and starting time was included for analysis of SSA. For variables 3 and 4, an extra factor product and its interactions with odour and exposure time were included. Product consisted of levels banana products, sweet products and savoury products for variable 3 and levels banana milkshake and meat soup for variable 4. The random part of the model consisted of random effects for sessions and participants, whereas correlations between repeated measurements at 1, 5 and 9 min were modelled by using a first order autoregressive correlation structure. For variables 3 and 4, an unstructured covariance matrix was added to account for correlations between scores for specific products. Correlations and residual variances were allowed to be odour-specific for variables 1 to 4. Degrees of freedom were calculated according to the method by Kenward and Roger.[177] Results from natural and artificial banana odours were combined, if they were not significantly different.

Before analysis, general appetite was calculated as the mean of hunger and desire-to-eat scores. General appetite and intensity were logit transformed by using $\ln[(y/100+0.01)/(1-y/100+0.01)]$. Change scores were calculated as after (t = 1, 5 or 9 min) minus before (t=0) exposure scores. SSA was calculated as the mean change in appetite for banana products minus the mean change in appetite for the reference products. For the change in appetite for banana products, change scores were averaged over all banana products. Similarly, the ratings for sweet and savoury products were averaged. The change in the expected pleasantness was considered a proxy measure for SSA, lacking subtraction of the mean change in the expected pleasantness for the reference products, because the latter is estimated to be approximately zero.

Statistical analyses were performed with SAS (version 9.1.3; SAS Institute). All results are shown as estimated means ± SE by using a mixed model unless stated otherwise. Results in the text on transformed data were back-transformed to the original scale. P-values <0.05 (two-sided) were considered significant.

Results

General appetite

Mean rated general appetite was 83 mm for natural banana, 82 mm for artificial banana and 85 mm for no-odour. Averaged over all conditions, the general appetite scores increased by 3 mm from 1 to 9 min of exposure (P<0.001). The effect of condition on general appetite was not significant (P=0.06) and did not significantly change over time (interaction between condition and time: P=0.27). General appetite did not differ between conditions at baseline (t=0) (P=0.65) and tended to peak at around 12.30 h (P<0.001).

Sensory-specific appetite (SSA)

SSA increased by 11.5 ± 2.3 mm (mean ± SD) during actively sniffing natural or artificial banana odour (P<0.001). The mean change in appetite for the reference products was -0.6 ± 7.8 mm (mean ± SD). SSA did not significantly change over time (P=0.65) and nor did condition and time interact (P=0.47). There were no differences in SSA between natural and artificial banana odour (P=0.22).

Appetite for banana, sweet, and savoury products

The change in appetite for banana/sweet/savoury products did not change over time (Figure 3.2; Table 3.1), but there was an interaction between condition and product. Compared with no-odour, exposure to natural and artificial banana odour increased the appetite for banana products (P<0.001) and sweet products (P=0.003), whereas the appetite for savoury products decreased (P<0.001). Natural and artificial banana odour did not differ in their effects on appetite for banana products (P=0.50), sweet products (P=0.69) or savoury products (P=0.28).

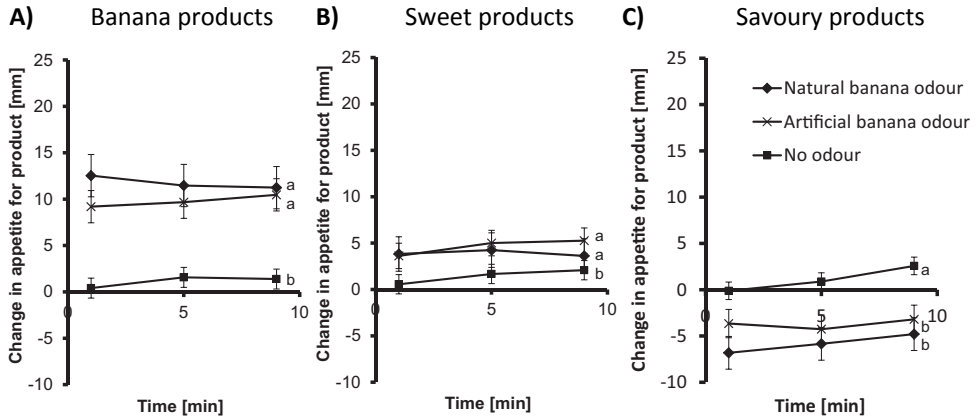

Figure 3.2 Changes in appetite for **(A)** two banana products, **(B)** three sweet products, and **(C)** three savoury products during exposure to natural banana odour (n=61), artificial banana odour (n=61) and no-odour (n=117), in normal-weight women, measured by using 100 mm visual analogue scales. Values are means ± SE. Labelled means per panel (averaged over 1, 5, and 9 min) without a common letter differ, P<0.05.

Table 3.1 F (with df) and P-values of all fixed factors for changes in appetite and expected pleasantness.

Factor	Change in appetite for banana/sweet/savoury	Change in expected pleasantness of banana milkshake/meat soup
Condition	$F_{2,177}$=4.5; *P*=0.013	$F_{2,175}$=4.1; *P*=0.019
Time	$F_{2,424}$=1.6; *P*=0.19	$F_{2,425}$=5.5; *P*=0.004
Condition x time	$F_{4,350}$=0.2; *P*=0.92	$F_{4,348}$=0.6; *P*=0.65
Product	$F_{2,220}$=30.9; *P*<0.0001	$F_{1,238}$=44.6; *P*<0.0001
Condition x product	$F_{4,204}$=17.2; *P*<0.0001	$F_{2,177}$=25.0; *P*<0.0001
Time x product	$F_{4,409}$=1.1; *P*=0.36	$F_{2,360}$=0.7; *P*=0.50
Condition x time x product	$F_{8,392}$=0.9; *P*=.054	$F_{4,302}$=1.4; *P*=0.23

Expected pleasantness ratings for banana shake and meat soup

Results revealed an interaction between condition and product (Figure 3.3; Table 3.1). Exposure to natural and artificial banana odour increased the expected pleasantness scores for banana milkshake (P<0.001) and decreased the scores for meat soup (P<0.001) compared with no-odour. There were no significant differences between natural and artificial banana odour on the ratings for banana shake (P=0.83) or meat soup (P=0.58). The expected pleasantness ratings increased over time (P=0.004). The 13-mm increase in the expected pleasantness of banana milkshake is considered a proxy value for SSA.

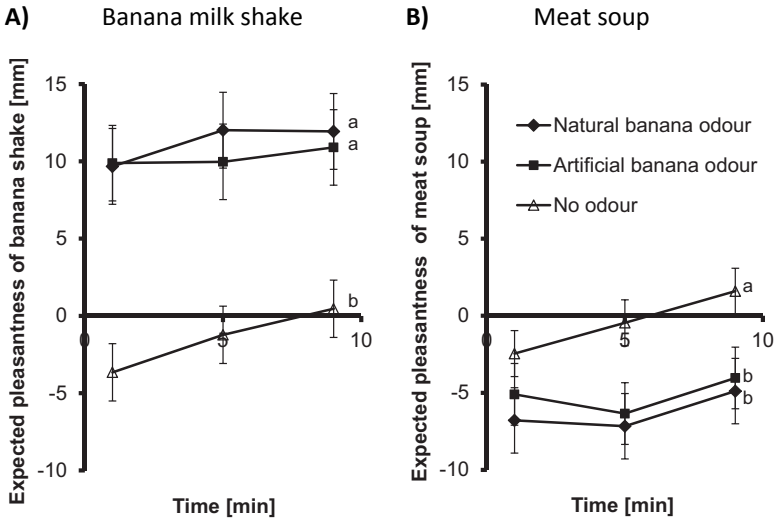

Figure 3.3 Expected pleasantness ratings for **(A)** banana milkshake and **(B)** meat soup during exposure to natural banana odour (n=61), artificial banana odour (n=61) and no-odour (n=117), in normal-weight women, measured by using 100 mm visual analogue scales. Values are means ± SE. Labelled means per panel (averaged over 1, 5, and 9 min) without a common letter differ, P<0.05.

Food intake

Exposure to banana odours had no significant effect on the *ad libitum* intake of banana milkshake (P=0.68). A quadratic effect of starting time that specifies when a participant performed the experiment was found on food intake (P=0.004). Food intake tended to peak around 13.00 h. Initial appetite at baseline (t=0) had no significant effect on intake (P=0.09). Intake of banana milkshake was 333 ± 29 g after smelling artificial banana, 343 ± 29 g after natural banana, and 351 ± 29 g after no-odour.

Odour intensity

The mean rated intensity was 86 mm for artificial banana, 78 mm for natural banana and 7 mm for no-odour. At every time point, rated intensity was greater in the banana odour conditions compared with no-odour (all P<0.001). Artificial banana odour was rated as more intense than natural banana odour (P=0.001). Furthermore, intensity decreased by 10 mm from 1 to 9 min of exposure in the banana conditions (P<0.001), which indicates that there was adaptation over time.

Discussion

The present study investigated the effect of actively sniffing banana odours on SSA, general appetite, and *ad libitum* intake. Actively sniffing banana, both natural and artificial, increased the appetite for banana products, relative to the appetite for the reference products (SSA) and decreased the appetite for savoury products. The circa 12-mm increase in SSA in the present study did not change during the 10-min active exposure, independent of using the appetite for specific product ratings or the expected pleasantness ratings. We consider the increase in the expected pleasantness compared with baseline to be a proxy value for SSA. SSA was determined with pleasantness and appetite ratings to explore potential differences in SSA caused by differences in liking versus wanting.[2] Other studies also reported an increase in the preference or craving for the cued food by using passive odour exposure.[36, 39, 40] Rolls and Rolls[57] on the other hand, reported a decrease in the pleasantness of the odour of the food after actively smelling food odours, which they termed olfactory SSS. However, the pleasantness of a food odour may develop differently during odour exposure compared with the pleasantness of a food as assessed in the present study. In addition, an increase in SSA of circa 10 mm was also found in our previous study by using passive exposure to banana odour.[179] The present study was executed under similar conditions as in our previous study, with a similar test procedure, appetite questionnaire, inclusion and exclusion criteria and odours.[179] Therefore, we find it legitimate to compare the current results during active smelling with the previous results during passive smelling. Results from the present and previous study,[179] suggest that the manner of odour exposure, active or passive, does not affect the development of SSA over time.

The mechanism responsible for the increase in SSA after smelling food odours, actively or passively, may differ from the mechanism that causes the development of SSS after modified sham feeding, although in both cases no food is actually ingested. During solely smelling food odours, such as in the present study and the study by Fedoroff *et al.*,[36] anticipation of forthcoming food intake may keep the appetite for the smelled food high. During a real meal, the appetite for a particular food is stimulated by the first bite[184] and diminishes over time.[11, 12] With modified sham feeding, however, the appetite for the tasted food decreases in a similar way as during real eating, but without the caloric intake.[57, 141-143] Modified sham feeding includes oral exposure, which may misinform the body by the pretence of real food intake. According to Morewedge *et al.,*[185] there is a great overlap in brain responses between the perception of food during real consumption and the mental imagery of consuming food. They demonstrated that repeatedly imagining eating M&M's or cheese cubes specifically decreased subsequent intakes of the imagined

food compared with other foods, which is a response similar to actually ingesting those foods. On the contrary, they also showed that repetitive imagining of moving the M&M's increased the subsequent intake of M&M's,[185] which is called sensitization and may parallel the increase in appetite for the cued food during food cue exposure (SSA). Anticipation of food intake during odour exposure is perhaps a different type of food cue than the imagination of food intake. The anticipation of future food intake during active or passive odour exposure may increase the appetite for cued foods, whereas the suggestion or belief that food intake had taken place (by mental imagery, modified sham feeding or real intake) may decrease the appetite for cued foods.

In addition to the specific increase in the appetite for banana products, actively smelling banana odours increased the appetite for sweet products and decreased the appetite for savoury products, as previously demonstrated.[37, 179] The categorization of food odours into sweet and savoury may be of biologic relevance.[179] Savouriness is often related to protein and salt content and sweetness to sugar content.[28, 31, 186] Therefore, food odours may communicate information about the (macro)nutrient composition of the associated foods as well[179, 187, 188] and help our body prepare for the intake of specific foods.[43, 173] The importance of these odour-induced changes in appetite for sweet and savoury foods on a behavioural level, i.e. actual food choice and food intake, remains to be further investigated.

Subjective hunger and appetite ratings often predict food intake,[e.g. 27] although not in every study.[e.g. 189] Therefore, both appetite ratings and food intake were measured in the present study. The *ad libitum* intake of the banana milkshake and general appetite were not affected by actively smelling banana. Other studies reported conflicting results that showed an increase,[33, 38, 44, 45] decrease[33, 44, 46] or no effect[37, 45, 47] of cue exposure on food intake. General appetite decreased by circa 4% after actively smelling dark chocolate[121] and hunger increased after active exposure to sight, smell and taste of palatable food.[33, 48] By using passive exposure to food odours, our previous study demonstrated a significant increase of 4 mm in general appetite,[179] whereas Coelho *et al.*[46] found no effects of passively smelled odours. The inconsistency in the results on general appetite and food intake most likely reflects the small effect sizes and reveals the complexity of food cue reactivity with several confounding factors, as explained below.

First, responses to food cues may depend on individual traits such as (un)restrained eating,[33, 47, 190] overweight/normal weight[44] and impulsivity.[45] Another confounding factor may be the similarity of the test food with the food cue, which may affect the relation between food cue exposure and intake or general appetite,[179] as also suggested by

Fedoroff *et al.*[36] Furthermore, the effect size of the response to food cues may depend on the type of food cue, i.e., smell, taste, sight, and/or thought.[149, 170] Reactivity to food cues is higher when people are hungrier[81, 83] and possibly depends on palatability.[175, 191] Altogether, the effect of food cues and odours on general appetite and subsequent *ad libitum* intake remains unclear and needs further investigation with consistency in choice of cues and foods and detailed participant characterization.

The difference between natural and artificial banana odour probably had no effect on the outcomes of the present study, because they both successfully represent banana products. Furthermore, the impact of the slight imbalance in the assignment of the treatment order was considered negligible because it was incorporated in the analysis.

In conclusion, SSA scores during 10 min of active sniffing were consistently high, with no decrease in SSA over time. These SSA scores are identical to the SSA found in a similar study that used passive smelling, suggesting that SSA is independent of the manner of sniffing and independent of exposure time. Moreover, actively sniffing banana odours increased the appetite for congruent sweet foods and decreased the appetite for incongruent savoury foods. The sweet/savoury categorization may suggest that food odours communicate information about the nutrient composition of their associated foods. Furthermore, sniffing banana odour did not affect food intake and general appetite. The inconsistency in results on food intake and general appetite, in the present study and in the literature, may reveal the complexity of food cue reactivity with many confounding variables. Future research could investigate the importance of the changes in SSA for actual food choice or food intake with the use of consistency in choice for cues and foods and detailed participant information.

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Chapter 3B

Orthonasal active odour exposure

Food preference and appetite after switching between sweet and savoury food odours

Submitted for publication. Mariëlle Ramaekers, Gerrit Gort, Catriona Lakemond, Martinus van Boekel, Sanne Boesveldt, Pieternel Luning

Abstract

Background: Exposure to food odours increase the appetite for congruent foods and decrease the appetite for incongruent foods, but the effects of exposure to a variety of food odours, as often occurs during daily life, is unknown.

Objective: Investigate how switching between sweet and savoury food odours affects the appetite for sweet and savoury products, food preference and general appetite.

Design: Thirty women (age: 18-45y; BMI: 18.5-25kg/m^2) actively smelled the contents of cups filled with banana, meat or water (no-odour) in a within-subject design with four conditions: no-odour/banana, no-odour/meat, meat/banana and banana/meat. In each condition, two cups with different fillings were smelled for five minutes after each other. Visual cues were obstructed. A switch from no-odour to meat or banana odour was considered as no-switch, whereas a change from meat to banana or banana to meat was considered as a switch.

Results: The currently smelled odour ($P<0.001$), as opposed to the previously smelled odour ($P=0.71$), determined the appetite for banana, sweet, meat, savoury and staple products, already 1 minute after a switch between sweet and savoury odours. Switching between odours did not significantly affect general appetite ($P=0.31$). The pleasantness of the odour decreased during odour exposure ($P=0.005$).

Conclusions: After a switch, the appetite for specific products quickly adjusted to the new smell and followed the typical pattern as found during odour exposure in previous studies. Interestingly, the appetite for the smelled food remained elevated during odour exposure, known as sensory-specific appetite, whereas the pleasantness of the odour decreased over time, known as olfactory sensory-specific satiety. This seeming contradiction may result from different mechanisms underlying the odour-induced anticipation of food intake versus the decrease in hedonic value during prolonged sensory stimulation.

Keywords: Food cue, Sensory-specific appetite, Olfactory sensory-specific satiety.

Introduction

Unhealthy eating habits such as unhealthy food choices or overeating increase the prevalence of obesity, diabetes, cancer, cardiovascular and other diseases.[1, 4, 5] Therefore, it is important to understand how various factors, for example sensory processes, influence our eating behaviour. Sensory processes play a role in food selection in several ways. First, associations between the nutrient composition and the sensory properties of foods, such as appearance, smell and taste, are formed due to repeated exposure in our daily lives.[28] These associations partly determine the pleasure that is derived from foods,[29, 30] whereupon pleasantness influences food selection. Moreover, these associations also facilitate the estimation of the nutrient composition of foods based on the sensory properties[28] and this information can be used for food selection, for example in case of nutrient deficits.[31, 32] Furthermore, recently consumed foods modulate food preference, which is likely driven by the need for variety. For example, the preference for savoury products decreases after eating a savoury meal, a phenomenon referred to as sensory-specific satiety.[11, 12] Finally, external factors such as exposure to sight, taste or smell of foods change our food preference.[33-37] It has been widely demonstrated that exposure to food cues increases the preference for the cued food.[36-40, 179, 192] For example, sweet odours increased the appetite for sweet products and savoury odours the appetite for savoury products.[179, 192] Moreover, sweet odours also decreased the appetite for savoury products and savoury odours for sweet products.[179, 192] The mechanism behind these findings has not yet been clarified.

This increase in appetite for congruent foods and decrease in appetite for incongruent foods may be caused by cephalic phase responses as previously suggested.[179] Food odours and other food cues elicit cephalic phase responses that prepare the body for the intake and digestion of foods.[42, 48, 193] In general, sweetness is associated with sugar content and savouriness with protein content,[28, 31, 186] with distinct routes of digestion for different macronutrients. Therefore, determining the type of food by exposure to food cues before ingestion, may prepare the body for the digestion of the specific macronutrients of the anticipated foods.[43, 179] It may be that once the body is prepared for the intake of a food with a certain (macro)nutrient composition, it is less favourable to ingest a food with a very different (macro)nutrient composition.[179]

In daily life though, for example by strolling a (super)market, exposure to a variety of food cues that prime for a wide variety of foods, may induce confusion in the body. Previous exposures to food cues may possibly interfere with exposures to new food cues. If our body indeed specifically prepares for the intake of cued foods, then it may take some time

to switch the appetite for (in)congruent foods according to the characteristics of new food cues.

To our knowledge, the effect of switching between different food cues on general appetite and food preference has not been investigated before. The objective of the current study was to determine how switching between sweet and savoury food odours affects the appetite for sweet and savoury products, food preference and general appetite. General appetite and the appetite for sweet and savoury products were measured at several time points during odour exposure to explore if possible changes after switching are immediate or take time. The results could provide insight in the processes behind the effect of food cues on food preference in real life.

Materials and methods

Experimental design

A within-subject design with the following four conditions was used: no-odour/banana, no-odour/meat, meat/banana and banana/meat. On each test day, a participant was exposed to one condition with two subsequent odour exposures and a one minute break in between exposures. Each odour was smelled actively for five minutes. Appetite measurements were taken at 1 and 5 minutes during exposure to the first odour and at 7 and 11 minutes during exposure to the second odour. The order of the conditions were balanced over the participants and as much as possible over the test days. Each participant was scheduled on four separate days, preferably once per week at the same time of the day between 11.20 h and 13.40 h. A switch from no-odour to meat or banana odour was considered as no-switch between odours, whereas a change from meat to banana odour or from banana to meat odour was considered as a switch (Table 3.2).

Participants

Thirty participants enrolled in the study with an average age of 21.6 ± 4.7 y and average BMI 21.9 ± 1.3 kg/m^2. Exclusion criteria were: dislike for banana, banana pie (Dutch pastry), steamed meat or beef soup (score < 5 on a nine-point scale), smoking, pregnancy or breast feeding during the last six months, lack of appetite, following an energy-restricted diet or change in body weight>5 kg during the last 2 months, hypersensitivity to any of the foods under study or being a vegetarian. It was explained to the participants that the influence of sensory signals on food choice was investigated. After the study, the participants were informed about the full study objectives. One participant missed the last session due to illness. All participants signed an informed consent form. All procedures

were in accordance with the Helsinki Declaration of 1975 (as revised in 1983). This study was registered at the Dutch trial register (NTR3830).

Table 3.2 Explanation of the terminology for previous exposure, current odour and switch in all conditions.

Condition	Time [min]	Previous exposure**	Current odour*	Abbreviation***
No-odour/banana	1, 5	Nothing	No-odour	N
	7, 11	No-odour	Banana	nB
No-odour/meat	1, 5	Nothing	No-odour	N
	7, 11	No-odour	Meat	nM
Meat/banana	1, 5	No sniffing	Meat	M
	7, 11	Meat odour	Banana	mB
Banana/meat	1, 5	No sniffing	Banana	B
	7, 11	Banana odour	Meat	bM

*Current odour is presently sniffed by the participants, which can be either the first or the second odour in a condition. **Previous exposure is the exposure that preceded the current odour. ***Abbreviation is the code used in the results section and defines the combination of previous exposure and current odour, with 'N': exposure to no-odour, 'M': meat odour, 'B': banana odour, 'nM': meat odour after no-odour, 'bM': meat odour after banana odour, 'nB': banana odour after no-odour, and 'mB': banana odour after meat odour.

Odours

Cups were filled with three different fillings, depending on the condition: 10 g of water in the no-odour condition, a tablespoon of medium ripe mashed banana or a tablespoon warm steamed meat (stoofvlees, Coertjens, Belgium). A tissue and a plastic lid were placed over the filling to prevent visual cues and odour contamination in the sensory room.

Measurements

An appetite questionnaire and food preference questionnaire were taken during the experiment.

The appetite questionnaire measured hunger and desire-to-eat over time on 100 mm computerized visual analogue scales (VAS, not at all – very).[21] Besides 'general' appetite, the appetite for 15 individual products was measured by using 100 mm VAS in a randomised order (for example, 'How large is your appetite for a banana at this moment?; not at all – very).[21] These products were divided into banana, meat, sweet, savoury and staple products. Banana and banana pie were selected as banana products, and bread roll with steamed meat and beef soup as meat products. Sweet products were mango, sweet pastry 'tompouce', strawberry yoghurt and M&M's. Savoury products were bread roll with

egg, tomato soup, cheese and salted peanuts. Staple products were bread bun, croissant and pancake. In addition, odour intensity (100 mm VAS, not at all – very) and feeling well (100 mm VAS, not at all – very) were added to the appetite questionnaire to check if the odours became overwhelming or affected the participants' well-being. Finally, odour pleasantness was monitored over time (100 mm VAS, not at all – very).

The food preference questionnaire (FPQ) was a computerized task as previously used in Ramaekers *et al.*[179] and based on work of Finlayson *et al.*[162, 163] (E-prime, v2.0; Psychology software tools, Sharpsburg, PA, USA) measuring food preference at the end of the 10 minutes of exposure to the odours. On each trial, two foods were simultaneously shown on a computer screen using digital colour photographs. The participants were asked to choose the food that they would like to eat most at that moment. The foods on the photographs were the same as the foods in the appetite questionnaire, plus additionally banana sweets, and little snack sausages. All banana products were compared against all non-banana products, including meat products. In addition, all meat products were compared against all non-meat products, leading to 84 comparisons. The frequency with which each product was chosen was determined.

Standardizing hunger state

The visits of each participant were scheduled at the same time of the day to standardize the individual hunger state. On the first test day, participants were instructed to consume a normal amount of breakfast, at least 2.5 hours before the start of the experiment. After this time, only water or weak tea were allowed. On the remaining test days, the participants were requested to consume the same breakfast as on the first test day. The diaries in which participants recorded their breakfast, changes in physical activity and health problems were checked for possible confounders and to increase commitment to the study rules.

Procedure

The participants took place in a sensory booth with two cups in front of them. Instructions were given on a computer screen (E-prime, v2.0). Participants first filled out the appetite questionnaire at baseline (t=0). Subsequently, they were requested to remove the plastic lid from the first cup, while keeping the tissue on the cup, and then intensely sniff the contents of the first cup. The appetite questionnaire was repeated 1 and 5 minutes after the start of the sniffing. The participants were encouraged by text on the computer to continue intense sniffing during the whole five minutes of odour exposure. Five minutes after the start of sniffing, the participants placed back the lid of the first cup and had a one-minute break. After that, they followed the same procedure with the second cup and

filled out the appetite questionnaire at t=7 and 11 minutes. After five minutes of intensely sniffing the contents of the second cup, participants placed back the lid and filled out the food preference questionnaire. At the end of the session, the participants had to choose between a banana or a bread roll with steamed meat that were placed on a plate in front of them. The participants were asked about their thoughts on the study objective at the end of the study in an end evaluation.

Data analysis

We studied the effects of previous exposure (i.e. odour switching), current odour, exposure time and their interactions on the following variables: general appetite (variable 1), odour pleasantness (variable 2), odour intensity (variable 3), feeling well (variable 4) and change in appetite for specific products (variable 5). Furthermore, the effect of condition (no-odour/banana, no-odour/meat, meat/banana and banana/meat) on food preference (variable 6), as measured with the food preference questionnaire (FPQ), was investigated. All variables were analysed with mixed linear models to account for the correlations between the repeated measurements.[183]

Before statistical analysis, general appetite was calculated as the average of the hunger and desire-to-eat scores. Change scores (post minus pre exposure) were averaged over all banana products for the mean change in appetite for banana products. Similarly, the ratings for meat, sweet, savoury and staple products were separately averaged. Variables 1 to 4 were logit transformed, using ln((y/100+0.01)/(1-y/100+0.01)), to stabilize the variance. Additionally, the FPQ data were transformed using arcsine(sqrt(frequency/max)), with max representing the maximum number of times a product could be chosen in a set. All comparisons between products in the FPQ were split into seven sets, with each set containing comparisons of two types of products. For example, the comparison between banana pie and beef soup belongs to the set 'banana-meat' (Table 3.5).

For variables 1 to 5, the fixed part of the mixed model consisted of the factor 'exposure group'. Exposure group was introduced being a combination of previous exposure (no sniffing, no-odour, switch), current odour (water, banana, meat) and exposure time (1, 5, 7, 11 min). Not all possible 3x3x4 combinations occurred together and therefore, we handled this incomplete factorial design by introducing the factor exposure group with 14 levels, representing the 14 actual combinations. For variable 5, an extra factor product (banana, meat, sweet, savoury, staple) and its interaction with exposure group was included. General appetite at baseline was used as covariate for variable 1. Variable 6 was analysed with fixed effect factors: condition, set and their interaction.

The random part of the mixed models consisted of random effects for sessions and participants. For variables 1 and 5, we used an autoregressive order-1 correlation matrix for the correlations among repeated measurements at 1-5-7-11 min and for variables 2-4 compound symmetry was used. Additionally, an unstructured covariance matrix modelled the (co)variances between scores for specific products for variable 5. For variable 6, an unstructured covariance matrix specified the (co)variances between sets. We were interested in comparisons between conditions per set. All degrees of freedom were calculated according to the method by Kenward and Roger.[177] Furthermore, correlations and residual variances were allowed to differ between current odours for variables 2, 3, 5.

The effects of exposure time (1 vs 5 and 7 vs 11 min), previous exposure, current odour, product and their interactions were analysed by comparing group means of exposure group, using contrasts. To investigate if previous exposure had an effect, B, nB and mB were compared against each other and M, nM and bM were compared against each other (Table 3.2). In case the effect of previous odour was not significant, the data from B, nB and mB were grouped as banana odour, M, nM and bM as meat odour and N as no-odour. When there was a significant effect of previous exposure, only the ratings at times 1 and 5 minutes were used for analysis of the effect of odour.

Statistical analyses were done with SAS (version 9.1.3; SAS Institute Inc., Cary, NC). All figures show mean values ± SD of the raw data. Results in the text are estimated means ± SE, using a mixed model. Results in the text on transformed data were back-transformed to the original scale to facilitate interpretation. P-values < 0.05 (two-sided) were considered significant.

Results

General appetite

There were no significant differences in general appetite between the four conditions at time=0 min ($F_{3,83.4}$=1.8; P=0.16) and general appetite did not change significantly over time (P=0.52; Figure 3.4; Table 3.3). The effect of previous exposure on general appetite was borderline significant (P=0.08) and therefore, the effect of odour was investigated only at times 1 and 5 min. There were no significant differences between meat, banana and no-odour at times 1 and 5 min (M vs B vs N; P=0.25). The interaction between previous odour and current odour was not significant ($F_{2,167}$=1.2; P=0.31).

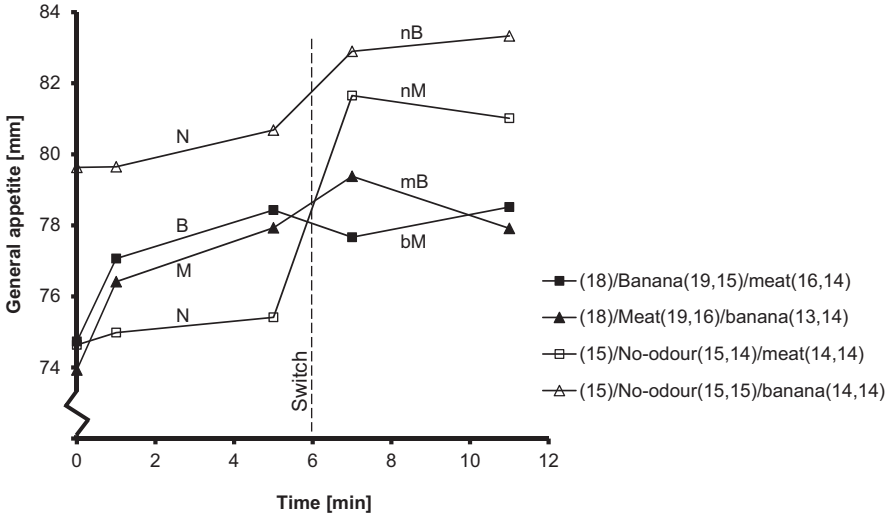

Figure 3.4 Mean general appetite scores during exposure to all conditions over time, measured by using 100 mm VAS. The numbers between the brackets represent the SD at respectively 0, 1, 5, 7 and 11 minutes. The dashed line represents the switch between odours. 'N': exposure to no-odour, 'M': meat odour, 'B': banana odour, 'nM': meat odour after no-odour, 'bM': meat odour after banana odour, 'nB': banana odour after no-odour, and 'mB': banana odour after meat odour.

Table 3.3 F-values with degrees of freedom and P-values of all factors with fixed effects.

	General appetite		**Odour pleasantness**		**Odour intensity**		**Feeling well**	
Fixed effects								
Exposure time	$F_{2,327}$=0.7	P=0.52	$F_{2,190}$=4.4	P=0.013	$F_{2,185}$=47	P<0.001	$F_{2,346}$=0.4	P=0.70
Previous exposure	$F_{4,196}$=2.1	P=0.079	$F_{4,68.4}$=0.3	P=0.88	$F_{4,91.5}$=0.7	P=0.60	$F_{4,206}$=0.6	P=0.68
Current odour	$F_{2,150}$=1.4	P=0.25[#]	$F_{2,98}$=47	P<0.001	$F_{2,107}$=103	P<0.001	$F_{2,230}$=6.0	P=0.003
ExpTime x Previous	$F_{4,324}$=0.7	P=0.58	$F_{4,128}$=0.4	P=0.79	$F_{4,128}$=1.0	P=0.40	$F_{4,346}$=2.0	P=0.097
ExpTime x Current	$F_{2,321}$=0.8	P=0.47	$F_{2,152}$=3.5	P=0.033	$F_{2,146}$=7 .7	P<0.001	$F_{2,346}$=1.2	P=0.32
Previous x Current	$F_{2,167}$=1.2	P=0.31	$F_{2,93.3}$=0.13	P=0.87	$F_{2,135}$=0	P=0.99	$F_{2,178}$=0.5	P=0.62
ExpTime x Prev x Curr	$F_{2,322}$=0.7	P=0.51	$F_{2,173}$=0.21	P=0.81	$F_{2,164}$=1.4	P=0.25	$F_{2,346}$=0.6	P=0.57
Ratings at baseline	$F_{1,124}$=250	P<0.001						

[#] Analysis at times 1 and 5 minutes only, because of possible interference of previous exposure.

Table 3.4 F-values with degrees of freedom and P-values of all factors with fixed effects for the change in appetite for specific products.

	Change in appetite for products	
Fixed effects		
Exposure time	$F_{2,336}$ =0.6	P=0.55
Previous exposure	$F_{4,281}$ =0.8	P=0.54
Current odour	$F_{2,303}$ =4.0	P=0.019
Product	$F_{4,254}$ =9.6	P<0.001
ExpTime x Previous	$F_{4,298}$ =1.2	P=0.32
ExpTime x Current	$F_{2,279}$ =2.6	P=0.073
ExpTime x Product	$F_{8,473}$ =1.1	P=0.38
Previous x Current	$F_{2,258}$ =0.7	P=0.50
Previous x Product	$F_{16,502}$=0.8	P=0.71
Current x Product	$F_{8,445}$ =16	P<0.001
All 3- or 4-way interactions		P>0.20

Appetite for banana, meat, sweet, savoury and staple products

Figure 3.5 shows the average changes in the appetites for banana, meat, sweet, savoury and staple products, further on named as the appetite for specific products. Previous exposure and its interactions with exposure time, current odour or product did not significantly affect the appetite for specific products (all P<0.05). Therefore, all ratings were grouped per current odour. The interaction between current odour and product was significant (P<0.001; Table 3.4) The changes in the appetites for banana, meat, sweet, savoury and staple products did not differ among each other in the no-odour condition (P=0.13), but differed during exposure to banana (P<0.001) and meat (P<0.001) odour. Exposure to banana odour increased the appetite for banana products (P<0.001), decreased the appetite for meat (P=0.026) and savoury (P=0.028) products and had no significant effect on the sweet (P=0.10) and staple products (P=0.48), compared with no-odour. Exposure to meat odour increased the appetite for meat (P<0.001) products, decreased the appetite for banana (P<0.001) and sweet (P<0.001) products and had no effect on the savoury (P=0.46) and staple products (P=0.72), compared with no-odour.

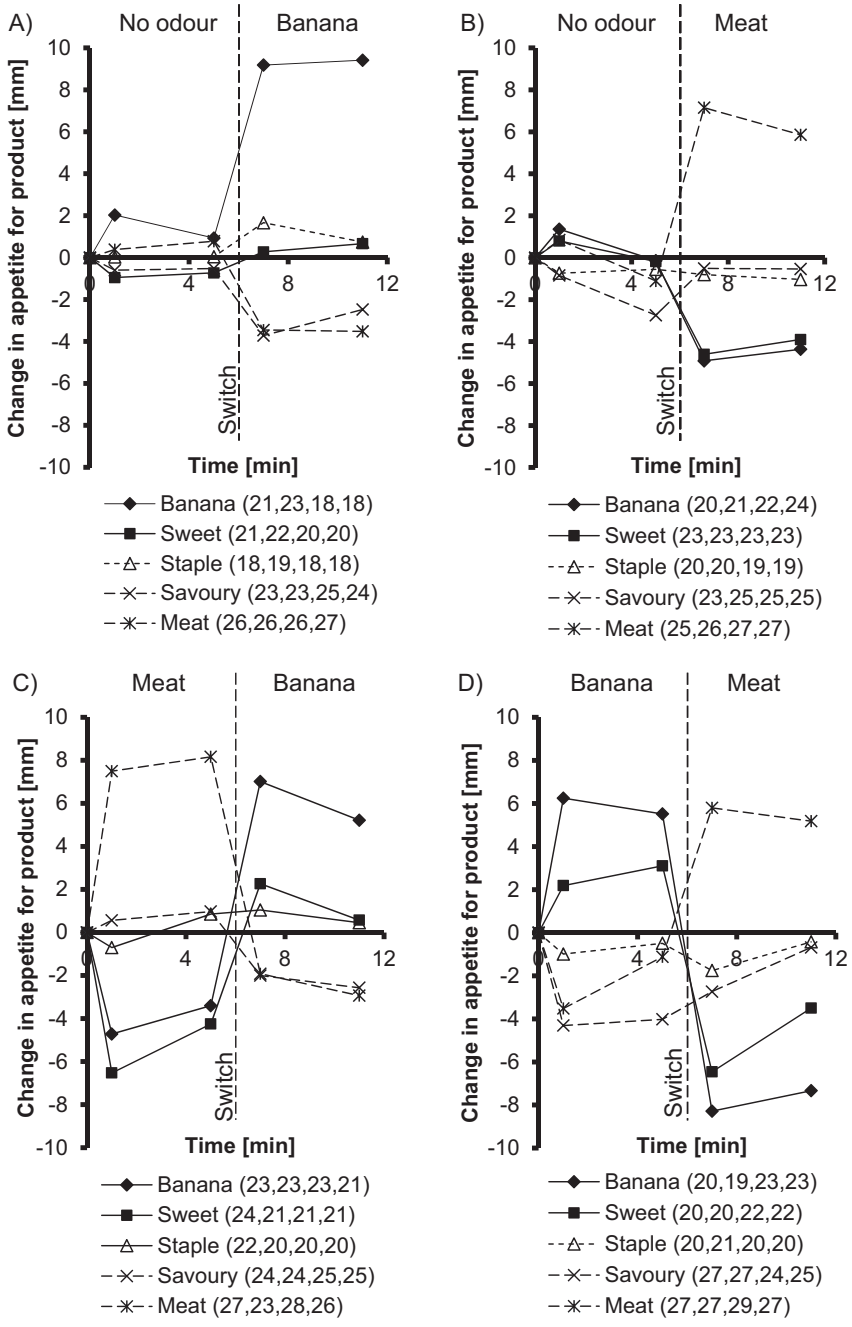

Figure 3.5 Mean change in appetite for banana, meat, staple, sweet and savoury products during exposure to **(A)** no-odour/banana, **(B)** no-odour/meat, **(C)** meat/banana and **(D)** banana/meat, measured by using 100 mm VAS. The numbers between the brackets represent the SD at respectively 1, 5, 7 and 11 minutes. The dashed line represents the switch between odours.

Preference for banana, meat, sweet, savoury and staple products, measured with FPQ

The preference of 84 pairs of food pictures was assessed, comparing banana and meat products against each other and against savoury, sweet and staple products (Table 3.5). When sets of banana and meat products were offered, the banana products were chosen more often when the last smelled odour was banana, than when the last smelled odour was meat (all P<0.05). In the banana-savoury and banana-staple sets, the banana products were more often chosen after the no-odour/banana condition, than after conditions no-odour/meat and banana/meat (all P<0.05). In the meat-sweet set, the meat products were chosen less often after condition no-odour/banana, than after conditions no-odour/meat and banana/meat (all P<0.05).

Table 3.5 Mean percentage of times a product was chosen per set of products, after exposure to different conditions, measured with the food preference questionnaire.

	Set						
Product 1	**Banana**-	**Banana**-	**Banana**-	**Banana**-	**Meat**-	**Meat**-	**Meat**-
Product 2	Meat*	Savoury*	Sweet*	Staple*	Savoury[#]	Sweet[#]	Staple[#]
Nr of comparisons	18	12	12	9	12	12	9
No-odour/banana	62[a]	60[a]	43	48[a]	46	33[a]	33
Meat/banana	58[a]	50[ab]	42	40[ab]	47	41[ab]	33
No-odour/meat	42[b]	47[b]	38	33[b]	53	56[b]	44
Banana/meat	42[b]	44[b]	43	33[b]	54	54[b]	46

Superscript with different letters denote significant differences at P<0.05

*Mean percentage of times that a **banana** product was chosen

[#] Mean percentage of times that a **meat** product was chosen

Actual food choice

After the odour exposure, thirteen participants always chose a banana and nine participants always chose a bread bun with steamed meat. Eight out of 30 participants switched their food choice between sessions. The choice for banana vs bread roll steamed meat was as follows: 19 vs 11 in no-odour/banana, 19 vs 11 in meat/banana, 12 vs 17 in no-odour/meat and 17 vs 13 in banana/meat.

Odour pleasantness

Figure 3.6 shows the pleasantness of the currently smelled odour. There was a significant interaction between current odour and time ($F_{2,152}$=3.5; P=0.033; Table 3.3). Ratings decreased on average by 4 mm from 1 min to 5 min exposure and from 7 minutes to 11 minutes during exposure to meat and banana odour (P=0.005). No effect of time was found during exposure to no-odour (P=0.61). Previous exposure had no significant effect on rated odour pleasantness ($F_{4,68.4}$=0.3; P=0.88). The pleasantness ratings of meat and banana odours were higher than of no-odour (both P<0.001). Differences between banana

and meat odour were borderline significant (P=0.08). Banana and meat odour ratings at 1+5 minutes were similar to ratings at 7+11 minutes (P=0.81).

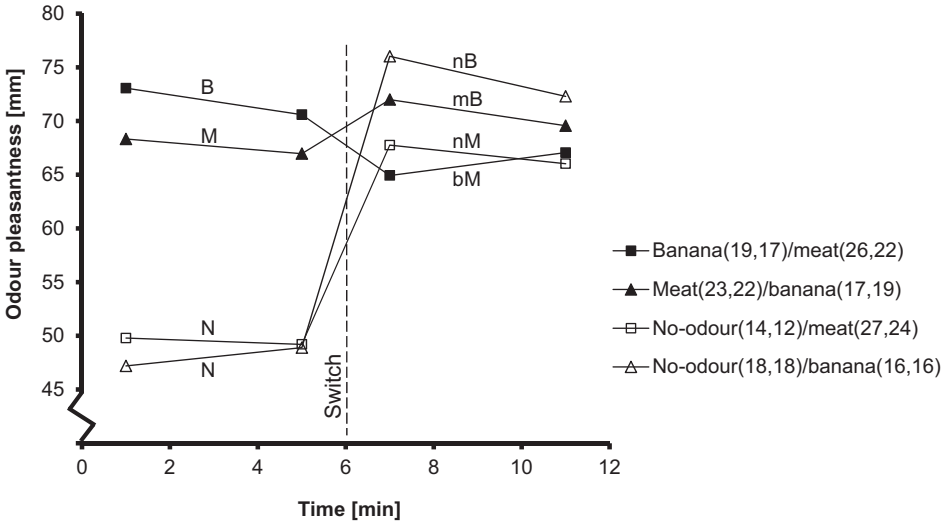

Figure 3.6 Mean odour pleasantness scores of the currently smelled odour in all conditions over time, measured by using 100 mm VAS. The numbers between the brackets represent the SD at respectively 1, 5, 7 and 11 minutes. The dashed line represents the switch between odours. 'N': exposure to no-odour, 'M': meat odour, 'B': banana odour, 'nM': meat odour after no-odour, 'bM': meat odour after banana odour, 'nB': banana odour after no-odour, and 'mB': banana odour after meat odour.

Odour intensity

Rated odour intensity was on average 77 mm for banana, 81 mm for meat and 15 mm for no-odour. The meat and banana odours decreased in intensity by on average 13 mm from 1 min to 5 min exposure and from 7 min to 11 min (P<0.001; Table 3.3). Previous exposure did not affect intensity ratings ($F_{4,91.5}$=0.7; P=0.69). The intensities of banana and meat odours were rated as higher than the intensity in no-odour (both P<0.001). Meat odour was rated 4 mm more intense than the banana odour (P=0.015). Banana and meat odour ratings at 1 and 5 min did not significantly differ from ratings at 7 and 11 min (B vs nB and M vs nM; P=0.20).

Feeling well

Feeling well ratings were on average 78 mm for banana, 79 mm for meat and 74 mm for no-odour. Feeling well was rated higher during exposure to banana or meat odour than during no-odour (P=0.013 and P<0.001 respectively). There were no differences between banana and meat (P=0.11).

Discussion

The objective of the present study was to investigate how switching between sweet and savoury odours affects the appetite for sweet and savoury products, food preference and general appetite. The results showed that the appetite for specific products adjusted within one minute to the currently smelled odour after a switch, with no lingering effect of a previously smelled odour. Apparently, our food preference system adapts within one minute to environmental changes (Figure 3.5). Interestingly, the pleasantness of the smelled odour decreased over time during sniffing (Figure 3.6), whereas the appetite for the smelled food remained elevated (Figure 3.5).

The present appetite and preference ratings for banana, sweet, meat, savoury and staple products followed the typical pattern as found during odour exposure in previous studies,[179, 192] regardless of switch. This pattern comprises the odour-induced increase in the appetite and preference for congruent foods and a decrease for the incongruent foods. The increase in appetite or preference for smelled foods was greater than the increase for other congruent foods.[179, 192] The present results, however, display a few exceptions to this pattern. The food preferences, measured at the end of the experiment with the FPQ, shifted towards products that are congruent with the last smelled odour (Table 3.5), but these preferences were less pronounced after exposure to the meat/banana condition than after no-odour/banana. Possibly, the preceeding meat odour slightly interfered with the banana odour, although the effect of previous exposure was not significant. Another deviation from the typical pattern is the lack of increase in the appetite for congruent sweet or savoury foods (VAS; Figure 3.5). In the present study, a different selection of sweet and savoury products was chosen in the appetite questionnaire than in our previous studies,[179, 192] which may have affected the results. Possibly not all savoury foods are congruent with the meat odour and not all sweet foods with the banana odour. Congruency with an odour may be a graded scale, with some products more congruent than others, depending on the associations of the odour with the products. Nevertheless, the appetites for the incongruent sweet and savoury foods consistently decreased during odour exposure in all studies.[179, 192] Therefore, it may be concluded that sweet products are evidently incongruent with savoury odours, however, the level of congruency within a sweet/savoury category may vary, i.e. savoury products can be more or less congruent with savoury odours depending on characteristics other than odour.

Interestingly, the present results revealed a decrease in odour pleasantness over time during smelling, even though the appetite for the smelled food remained elevated. Rolls

and Rolls[57] ascribed the decrease in odour pleasantness that was found in their study to olfactory sensory-specific satiety (olfactory SSS). Sensory-specific satiety is described as the decrease in pleasantness of, or desire-to-eat recently consumed foods, relative to uneaten foods.[11, 12] Also the lower intake of a similar food compared with a dissimilar food, after consumption of a preload, has been attributed to SSS.[12] The name olfactory SSS suggests a lack of appetite specifically for the smelled food, whereas our present and previous[179, 192] results showed an increase in the appetite for the smelled food during odour exposure, coined sensory-specific appetite (SSA).[179] These seemingly contradictory results are perhaps the consequence of different underlying processes that determine odour pleasantness and the appetite for the smelled food. Smelling foods initiates anticipation of food intake[42] and this anticipation may consequently elevate the appetite for the smelled food, as found in our present and previous studies.[179, 192] On the other hand, the decrease in pleasantness ratings during odour exposure is possibly caused by the actual stimulation of the chemical senses. Exposure to odours decreased the pleasantness of the odour that was perceived, but not the pleasantness of the taste of the smelled food that was not actually stimulated.[57] Repeatedly imagining eating M&M's decreased subsequent food intake, but had no effect on the pleasantness of the M&M's which indeed were not actually perceived by the senses.[185] During eating, the pleasantness of the odour and taste of the food decreases.[11, 57] Therefore, we hypothesise that actual stimulation of our senses, i.e. taste buds and olfactory receptor cells, causes a decline in the pleasantness of the perceived odour and taste. The present results indicate that the decrease in odour pleasantness during exposure underlies a different construct than the changes in the appetite for specific products, although until now both observations have been explained by the opposing terms SSS and SSA.

The banana and meat odour were rated as pleasant, even after the small decrease, and therefore smelling them probably still contributed to the enhancement of the appetite for those foods. The 4 mm decrease in odour pleasantness found during smelling in the present study was smaller than the 12 mm decrease that was found by Rolls and Rolls.[57] However, Rolls and Rolls[57] rated the pleasantness prior to, and after 5 minutes of sniffing, whereas our participants started rating after 1 minute. Possibly, odour pleasantness already decreased in the first minute of odour exposure.

Moreover, food preference adjusted to a new odour within the first minute. Food preference may change even within the first seconds after a switch in odours, because it is known that a few seconds of food odour exposure already elicit cephalic phase responses.[e.g.194] The current set-up did not allow for such quick measurements, because answering the appetite questions for a set of 15 products took 1 minute. We avoided

asking questions in the first minute, because we anticipated that changes would already take place within the first minute and we aimed to keep the circumstances under which the first and the last questions were answered the same as much as possible. The largest changes in food preference occur within the first minute after odour exposure or odour switch, after which it appears to remain stable.

At the end of a test session, participants received either a banana or a bread roll with steamed meat. Twenty-two of the 30 participants always chose the same product, regardless of odour exposure, which is likely caused by strong initial preferences.[195] In addition, the FPQ data also showed that around 20% of the choices between sweet and savoury products shifted depending on the set (banana-meat, banana-savoury and meat-sweet). Likely, odours are only able to change food choice when preference is ambiguous.

Finally, the present results do not support the suggestion that switching between odours affect general appetite. To our knowledge, this is the first study that investigated the effect of a switch between odours on general appetite or appetite for specific foods.

In conclusion, the appetite for specific products rapidly adjusts after a switch to the new odour and follows the typical pattern as found during odour exposure in previous studies. Surprisingly, there are no significant effects of previous exposure to odours. Interestingly, the pleasantness of the smelled odour decreases over time, whereas the appetite for the smelled food remains elevated during smelling. This seeming contradiction may result from different mechanisms, such as a decrease in hedonic value during prolonged sensory stimulation versus anticipation of food intake. Possibly, a gradual shift in food preference can be observed in the first minute after a switch between odours, when the set-up would allow for such quick measurements.

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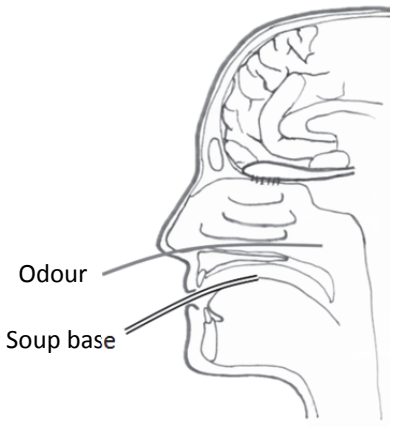
Conflict of interest

The authors declare no conflict of interest.

Chapter 4A

Retronasal odour exposure



Odour exposure time and odour concentration in relation to satiation

Based on 'Aroma exposure time and aroma concentration in relation to satiation.' *British Journal of Nutrition,* 2014; **111**(3). Mariëlle Ramaekers, Pieternel Luning, Rianne Ruijschop, Catriona Lakemond, Harold Bult, Gerrit Gort, Martinus van Boekel

Abstract

The present study investigated the effect of odour exposure time and odour concentration on *ad libitum* intake and subjective satiation. In a within-subject study, thirty-eight unrestrained, healthy female participants (age: 18–39 years; BMI: 18.5–26.0 kg/m^2) were asked to consume tomato soup during lunchtime, until they felt comfortably full. Every 30 s, the participants consumed 10 g of a bland soup base while tomato soup odour was delivered separately through the nose via a retronasal tube that was attached to an olfactometer. This gave the impression of consuming natural tomato soup. For each sip, the odour varied in exposure time (3 and 18 s) and concentration (5x difference), resulting in four different test conditions. *Ad libitum* food intake, appetite profile parameters and sensory-specific satiation were measured. A 9% lower food intake was observed when the participants were exposed to the condition with 18 s exposure time and a high concentration than when exposed to the other three conditions. These results indicate that changing the retronasal odour release by odour concentration and odour exposure time affects food intake.

Keywords: Food intake, Odour concentration, Odour exposure time, Sensory-specific satiation

Introduction

Understanding the factors that influence meal size can be helpful for finding strategies to limit overconsumption. It has been widely accepted that sensory processes play an important role in the development of satiation.[56, 168, 196-198] Satiation is the process that brings a meal to an end. Although Brunstrom and colleagues[153, 199] suggested that sensory properties might be more important for meal onset and meal planning than for meal termination, there are also strong indications that sensory processes influence meal termination and determine meal size; for example, sensory variety in a meal increases food intake.[12, 13, 58, 59, 141]

Another example is a lower *ad libitum* food intake after a longer oral exposure time per volume of consumed food.[52-54, 138, 200, 201] The decreases in food intake found in these studies varied between 9 and 30%. These studies focused on the effect of total flavour exposure time, which is a combination of odour, taste and mouthfeel. Ruijschop *et al.*,[123] however, focused on the unimodal effect of odour exposure time on satiation. The participants received a fixed preload of ten sips from a sweetened milk drink during a short or long odour delivery. Ruijschop *et al.*[123] found that an increase in exposure time to strawberry odour increased subjective satiation, measured by using visual analogue scales (VAS). The effects of sensory exposure time on food intake and subjective satiation were attributed to sensory-specific satiation (SSS). SSS is the decrease in the pleasantness of a food eaten to satiation, relative to uneaten foods.[12, 13] The subsequent *ad libitum* intake of a normal strawberry drink, in the study carried out by Ruijschop *et al.*,[123] showed no differences between the conditions. The previously mentioned increase in subjective satiation with an increase in odour exposure time[123] might reduce *ad libitum* food intake when measured directly during odour delivery in a different experimental set-up.

Besides the effect of sensory exposure time on satiation, researchers have investigated the relationship between flavour intensity and satiation/SSS. Flavour intensity is the perceptual consequence of a certain stimulant's concentration. The effects of flavour intensity on satiation are not consistent though. Vickers *et al.*[202] and Lucas and Bellisle[144] showed, for example, that people consumed less when given the better-liked high-sweetened yogurt than when given the low-sweetened yogurt. This tendency of people satiating more from products with high taste or flavour intensities has been observed in a number of other studies,[203-206] whereas others have reported no effects of flavour intensity[207-209] or have even found an opposite effect.[210] Chung and Vickers[210] found a lower SSS after drinking an optimal-sweet tea than after drinking a low-sweet tea. The inconsistency in the outcomes could be partially explained by the different test foods and

different methods used to assess satiation, such as *ad libitum* food intake, subjective appetite ratings on VAS and decrease in pleasantness to assess SSS. For example, participants drank 25 % less from the equally liked lemon-flavoured ice tea with the strongest flavour intensity (including sweet taste), but did not show differences in appetite ratings.[205] Moreover, in some studies, only taste intensity has been reported to vary,[144, 202, 204, 210-212] while others have focused on total flavour intensity, which includes both taste and odour.[203, 205-207, 209] So far, the unimodal effect of odour intensity on satiation has never been investigated. It is unknown whether an increase in odour concentration would lead to a lower food intake.

Odour volatiles are released from foods in the mouth while eating. After swallowing, these volatiles pass through the pharynx to the nasal cavity where they reach the olfactory epithelium. We refer to this pathway as retronasal odour stimulation, as opposed to orthonasal odour stimulation, which occurs when odorants enter by the inhalation of volatiles through the nose. The concentration of odour volatiles that is released over time during consumption of a single bite is referred to as the odour release profile. During food consumption, odour release profiles depend on food properties such as texture, temperature and composition[213-217] and also on human characteristics such as chewing behaviour, salivation and morphology of the nose.[218-220] Since the use of odours does not contribute to the energy density of foods, any suppressive effects of odour on food intake could, therefore, reduce energy intake.

The objective of the present study was to investigate whether retronasal odour concentration and/or odour exposure time affect satiation, measured as *ad libitum* food intake. We examined the effect of well-defined odour release profiles, presented retronasally by an olfactometer, on the development of satiation. Odours that are presented retronasally are processed differently than odours presented orthonasally.[112, 124, 125, 221] Especially, the pathway-specific contribution to the perception of taste[124] and mouthfeel[117] may add to the satiating properties of the odour. In order to verify a possible relationship between odour concentration and food intake, we maximised the differences in concentrations within the limits of acceptability. Besides food intake, we measured appetite profile parameters on VAS and we performed a traditional SSS test. We hypothesised that an increase in both odour concentration and odour exposure time increases SSS, which in turn increases subjective satiation and decreases *ad libitum* food intake.

Materials and methods

Participants

For the present study, healthy women aged 18–45 years and with a BMI of 18.5–26 kg/m^2 were recruited from the surrounding areas of Ede and Wageningen. Unrestrained eaters on the basis of the Dutch Eating Behavior Questionnaire (score <2.91)[159] and women who liked tomato soup (score >5 on a nine-point scale, reported in the online inclusion questionnaire) were included. Women who had followed an energy-restricted diet during the last 2 months, had change in body weight >5 kg during the last 2 months, were pregnant or breast-feeding during the last 6 months or had a lack of appetite for any reason were excluded. The olfactory function of the participants was tested using Sniffin' Sticks (Burghart Medical Technics) as described by Hummel *et al.*[222] The test consisted of an examination of odour threshold (n-butanol), odour discrimination and odour identification. Women with a total score <27 on threshold, discrimination and identification were also excluded. In total, forty-three women aged 24 ± 5 years and with a BMI of 22.5 ± 1.6 kg/m^2 were enrolled for the study. Due to reports of discomfort due to the retronasal tube, newly discovered pregnancy or dislike of the test products, five participants were excluded from statistical analysis. To reduce the number of missing data due to sickness, hay fever or misinterpretation of the instructions, eight participants came for an additional test session. The participants were unaware of the change in odour concentration and odour exposure time and were informed that the influence of taste and smell on tomato soup consumption was being investigated. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Medical Ethical Committee of Wageningen University. Written informed consent was obtained from all the participants.

Test products

Tomato soup was used as the model product, because it meets the criteria of being homogeneous, liquid, commonly consumed during lunch and familiar to the participants. A bland soup base (with little tomato odour) was given orally, with the well-defined retronasal odours being presented simultaneously (Figure 4.1), to investigate the unimodal effect of odour concentration and odour exposure time on *ad libitum* intake. The soup base and odour had to be of perceptually matching qualities, i.e. congruent, in order to be perceived as tomato soup. The soup base consisted of 5 g Maggi Bouillon (Nestlé, the Netherlands), 4 g Cup-a-Soup Tomato Crème (Unilever, the Netherlands), 30 g modified starch 'Honig allesbinder' (Heinz, the Netherlands) and 561 g cooked water. The soup base contained 96 kJ/100g energy. Batches of 600 g soup base were kept at 60 °C

using a water-bath. The soup was consumed at a temperature between 50 and 55 °C. The odour used was a mixture of three flavours (Givaudan) dissolved in water: 6 g tomato (RB-329-620-8) + 0.15 g pizza herb (UN-981-546-3) +0.2 g soup greens (CT-722-418-3) per 100 g solution.

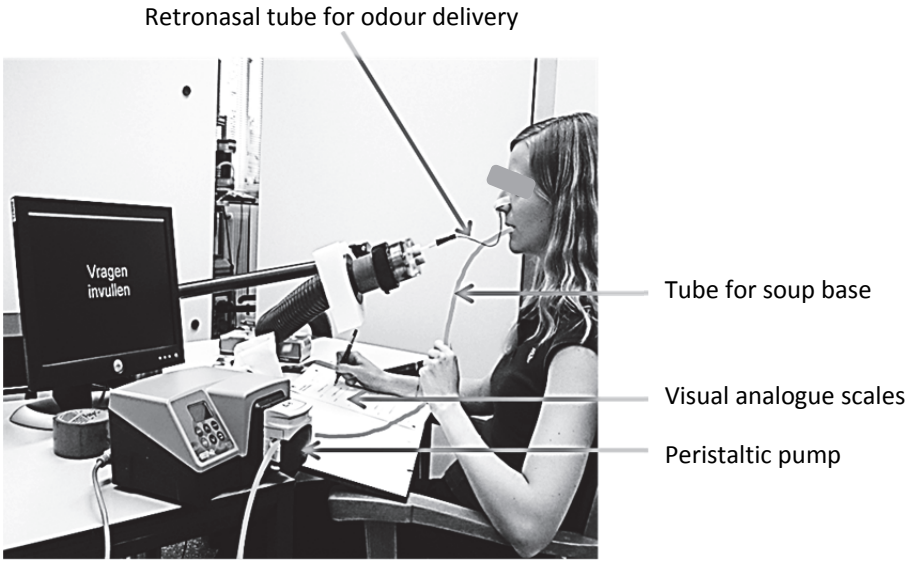


Figure 4.1 Experimental set-up with retronasal odour delivery via a retronasal tube that is connected to an olfactometer, in combination with delivery of soup base via a tube using a peristaltic pump.

Development of retronasal odour release profiles

The 'natural' odour release during regular soup consumption was measured in vivo, with atmospheric-pressure chemical ionisation–mass spectrometry as described previously by Ruijschop *et al.*[123] During the full scan, the response of all compounds with molar masses between 50 and 250 g/mol was determined. The ions with molar masses 80, 100 and 148 g/mol gave the highest response during the 'full scan' and were selected for measuring odour release. Odour profiles that were presented retronasally during soup consumption experiments were based on these measured odour release profiles.

Four different retronasal odour release profiles were generated, using a computer-controlled four-channel olfactometer based on air-dilution olfactometry (OM4, Burghart, Wedel, Germany). This allowed full control of the odour release profiles independently of food properties and human characteristics. The odour release profiles differed in concentration and length and were coded as 'low-short', 'low-long', 'high-short' and 'high-long'. The chosen exposure times were either 3 s (short) or 18 s (long). Moreover, these profiles were derived from the measured odour release profiles by decreasing their

concentration to create the 'low'-odour release profiles and by increasing their concentration to create the 'high'- odour release profiles. Differences in concentrations were chosen in such a way that four colleagues at NIZO food research perceived the lowest concentration as a weak intensity and the highest concentration as strong but not unpleasant or unnatural. This was done to maximise the effects of odour concentration on intake within the limits of natural soup odour compositions. The differences in concentrations were achieved by varying the duration of the odour pulses that were initiated every second. Accordingly, the odour pulse of the low odour concentration was five times shorter than that of the high odour concentration, but the pulse patterns over time of 'low' and 'high' concentrations were the same. At the chosen olfactometer flow rate and pulsation frequency of 1 Hz, the odour pulses blend into a continuous percept that has an intensity proportional to the average odour concentration.

The olfactometer was set at a constant dilution rate by mixing 0.5 litres/min of odorised air with 7.5 litres/min clean humidified air, resulting in a constant odourised air flow of 8 litres/min. Odour pulses were generated by switching between odourised and non-odourised air while keeping the overall flow rate constant. At the chosen flow rate, this resulted in a stimulus rise time <20 ms. The odour solution was refreshed every 2 minutes (after every fourth sip) to reduce the depletion of volatiles from the odour vessel of the olfactometer (Figure 4.2B). Subsequently, the four odour release profiles, as presented to the respondents, were verified by connecting the olfactometer to the atmospheric-pressure chemical ionisation– mass spectrometry equipment. For each condition, twelve odour release profiles were measured (Figure 4.2).

Experimental design

We used a randomised 2 x 2 within-subject design, investigating the effects of odour exposure time (3 and 18 s) and odour concentration (low and high). In total, the participants visited the test location on five separate days, with a washout period of at least 5 d. Before the actual experiment, on a separate day, the participants were tested on odour sensitivity and informed individually about the experimental procedure. On the other 4 d, the participants were exposed to one of the four odour release profiles. The order of the conditions was randomised over the participants in such a way that the conditions were spread over test days and sessions as much as possible. Although Ruijschop *et al.*[122, 123, 219] did not observe any effect of sessions on the results in similar previous experiments, results of the present study indicated that the participants had to get accustomed to the experimental setting. Therefore, the results obtained for the first session were not used in the data analysis and the session was considered a training session.

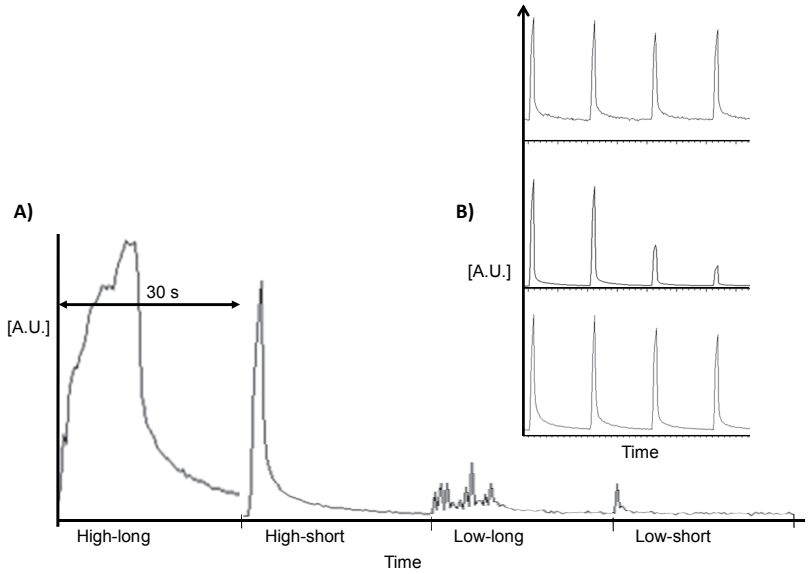


Figure 4.2 Odour release profiles measured with atmospheric-pressure chemical ionisation–MS. **(A)** Odour release curves for the four test conditions, measured with molar mass 80 g/mol. **(B)** Odour release curves of the 'high-short' condition showing depletion during four sips, measured with compounds with molar masses 80, 100 and 148 g/mol. A.U., arbitrary units.

Procedure

The participants were instructed to consume the same breakfast on all the test days and record their food intake in a diary to standardise the individual state of hunger. To ensure that they arrived in a hungry state, they were not allowed to eat or drink, except for the consumption of beverages containing no energy, the last 3 h before the start of a test session and nothing at all 1 h before the start of a session. The participants were tested between 10.40 h and 14.20 h.

After arrival, the participants first completed the appetite questionnaire and performed the first part of the SSS test (detailed explanation in the next section). After that, a medically trained person inserted a silicon suction catheter with a total length of 20 cm (CH 10; D-Care B.V. Houten, The Netherlands; further on referred to as 'retronasal tube') into the lower meatus of one of the two nasal cavities with the outlet positioned at the epipharynx of the soft palate, approximately 7.5 cm from the naris.[112] The retronasal tube was then connected to the olfactometer. The participants could breathe normally. Furthermore, it was desirable to have enough time in between odour stimulation to keep adaptation as low as possible, while also a normal eating rate was preferred. The time in between odour stimulation was set to either 27 or 12 s, depending on the test condition (3 or 18 s exposure time, respectively). In this way, the amount of odour adaptation due to

frequent exposure could be reduced. This resulted in an eating rate that was four times lower than the average eating rate for soup.[223]

Every 30 s, the participants swallowed one sip (10 ± 0.04 g) of soup base. During soup consumption, the participants received instructions on a computer screen and heard beeps notifying them when a sip of soup would come into their mouth, when to swallow and when to complete the appetite questionnaires. Software that has been described previously[117] steered the olfactometer, the peristaltic pump, the beeps and the instruction screen. The participants received the soup base into their mouth through a silicon tube (diameter 4.8 mm, Rubber B.V., Hilversum, The Netherlands) by means of an electric peristaltic pump (Watson-Marlow, Bredel, USA). To ensure temporal synchronisation of oral and nasal stimuli to facilitate sensory integration of the oral soup stimulus and the retronasal odour,[117, 224] the participants were subjected to odour exposure just before or at the instructed moment of swallowing. This resulted in a realistic impression of consuming tomato soup.

The participants were instructed to consume tomato soup until they felt comfortably full. At that moment, they had to inform the experimenter, who would stop the system. All the participants had to stay in the test set-up with the retronasal tube in the nose for a minimum of 25 minutes to prevent meal termination due to inconvenience or boredom. After meal termination or 25 minutes, the retronasal tube was removed and the second part of the SSS test filled out.

Data collection

The computer recorded the number of sips to determine the *ad libitum* soup intake. Furthermore, ratings of hunger, satiation, fullness, desire-to-eat, appetite for something savoury, appetite for something sweet and thirst were recorded on 100 mm VAS (not at all – very much) before (baseline), during and after food intake. Participants completed the appetite questions during and after food intake at ten fixed time points (4, 8, 12, 16, 20, 25, 30, 35, 40 and 50 minutes after the start of food intake) and one directly after finishing consumption. The pleasantness of the soup was evaluated at the same time points during soup consumption. Additionally, after three sips, the participants gave an initial judgement of the soup by rating pleasantness (not at all – very much), overall flavour intensity (not intense – very intense) and length of aftertaste (not long – very long) on 100 mm VAS. Intensity was measured with VAS to collect normally distributed data.[225] Further, eleven foods on pictures were evaluated on expected pleasantness and desire-to-eat (DTE) on 100 mm VAS (not at all – very much) before and just after the food intake to calculate SSS. The pictures represented a bowl of tomato soup, glass of tomato juice, plate

with pasta and tomato sauce, fresh tomatoes, ginger bread, Gouda cheese, strawberry drink, croissant, boiled eggs, hot chocolate milk, and apples. All questionnaires were filled out on paper and scanned using TeleForm (v 10.1; Cardiff, USA).

Data analysis

Statistical analyses were carried out using SAS (version 9.1.3; SAS Institute, Inc., USA). Unless stated otherwise, two-sided tests were used. P-value <0.05 was considered significant. Raw data are presented as means and standard deviations and model results as least-squares means and standard errors of the least-square means. The latter are estimated means, based on a mixed model adjusted for covariates and random effects and further on referred to as means and standard errors. We considered the first session as a training session and excluded data obtained in this session from the analysis. A slightly unbalanced dataset was obtained.

Differences in *ad libitum* intake between the test conditions were compared using a mixed model fitted by restricted maximum likelihood (proc mixed, SAS). Mixed models can handle missing and unbalanced data.[183, 226] The *ad libitum* intake was the dependent variable with treatment factors concentration and exposure time, order (=session) as a block factor with a fixed effect, maximum pleasantness as a covariable and participant as a random variable. Order was included in the model, because food intake tended to increase with the number of completed sessions. The maximum rated pleasantness was included, because people tend to consume more when given more-pleasant foods. The error variances were allowed to be different between the sessions, because the participants tended to become accustomed to the set-up, resulting in decreasing variances over time. We first tested for overall differences among the four test conditions. Subsequently, we split results into main and interaction effects of concentration and exposure time. Differences in intake due to test conditions were compared using *post-hoc* t-tests with Bonferroni correction. One-sided tests were used for comparison between the test conditions, because we expected a lower food intake during a longer exposure time and/or a higher concentration.[123] Some of the participants gave exceptionally low pleasantness scores for the soups. To evaluate the influence of these data on the outcome of the study, we reanalysed the data after removal of all the data with maximum pleasantness scores <45.

The number of appetite and pleasantness questionnaires that the participants filled in during soup consumption varied among the participants and test conditions, because they stopped eating at different moments. At baseline (t=0), there were 118 observations with complete appetite questionnaires (initial ratings). Of the 118 observations, 113 were left

8 minutes after the start of *ad libitum* intake, while 98 were left after 12 minutes and 71 after 16 minutes. The change in appetite and change in pleasantness were calculated by subtracting the initial ratings from the ratings after 12 minutes of consumption. Differences in 'change scores' between the test conditions were compared using a mixed model. The change scores of appetite and pleasantness ratings were the dependent variables with treatment factors concentration and exposure time, order as a block factor with a fixed effect, initial ratings as a covariate and participant as a random variable. The error variances were allowed to be different between the sessions.

The change in pleasantness and DTE of the foods presented on pictures were calculated by subtracting the pre-consumption ratings from the ratings after food intake to determine SSS. Subsequently, the average change scores of the tomato soup (eaten) were compared with the average change scores of the non-tomato products (uneaten), by using a mixed model. The change scores of the pleasantness and DTE ratings were the dependent variables, with (un)eaten food as treatment factor, session as block factor with a fixed effect, and participant as random variable. The error variances were allowed to be different between sessions. Similarly, the change in tomato products were compared with non-tomato products and savoury products were compared with sweet products.

Results

Dataset

Data obtained in the first session were removed before data analysis. Split up per condition the dataset contains data from thirty participants in the 'low-short' condition, thirty-two participants in the 'low-long' condition, twenty-nine participants in the 'high-short' condition and twenty-seven participants in the 'high-long' condition. A slightly unbalanced dataset with repeated measurements on thirty-eight participants and in total 118 observations was collected.

Odour release profiles

For each condition, twelve odour release profiles were generated by the olfactometer and measured using atmospheric- pressure chemical ionisation–MS. The average maximum concentrations of the odour release profiles in the four conditions were determined, which were greater in the 'high' than in the 'low' odour release profiles. The difference in maximum concentration between the 'high' and 'low' odour release profiles was sixteen times for components with molar mass 80 g/mol, fourteen times for components with molar mass 100 g/mol and six times for components with molar mass 148 g/mol. The duration of the 'long' conditions was indeed longer than that of the 'short' conditions

(Figure 4.2A). Furthermore, the concentration decreased over time due to the depletion of the odour solution (Figure 4.2B), but this was not the same for the three different volatiles that were measured. Between the first and the fourth sip, the average depletion was 0% for compounds with molar mass 148 g/mol, 14% for compounds with molar mass 80 g/mol and 74% for compounds with molar mass 100 g/mol. Over all the conditions, the mean intensity was 54 ± 20 and the mean aftertaste was 45 ± 20. Neither rated intensity nor rated aftertaste was affected by exposure time, concentration, or the interaction between exposure time and concentration (all P>0.05).

Ad libitum intake

The mean *ad libitum* intake was 388 ± 175 g of soup with the 'low-short' odour release profile, 368 ± 154 g of soup with the 'low-long' profile, 350 ± 135 g of soup with the 'high-short' profile and 333 ± 144 g of soup with the 'high-long' profile (Figure 4.3). The effects of exposure time ($F_{1,73}$=3.59; P=0.062), concentration ($F_{1,73}$=3.90; P=0.052) and the interaction between concentration and exposure time ($F_{1,73}$=2.87; P=0.095) were not significant, although an overall effect of test conditions ($F_{3,73}$=2.96; P=0.0379) was found. Both maximum pleasantness ($F_{1,73}$=5.16; P=0.026) and order ($F_{1,73}$=11.12; P<0.0001) contributed significantly to the statistical model. The standard deviation due to interperson variability alone was equal to 132 g. Although the main and interaction effects were not significant, the overall F-test showed that there were differences between the test conditions. Therefore, we carried out post-hoc t-tests with Bonferroni correction. Results showed that the participants consumed less in the 'high-long' condition than in the other three conditions. The relative decreases in intake as calculated from the mixed model results were 9.1% (P=0.044; one-tailed) between the 'high-short' and 'high-long' conditions, 9.3% (P=0.035; one-tailed) between the 'low-long' and 'high-long' conditions, and 9.4% (P=0.029; one-tailed) between the 'low-short' and 'high-long' conditions. No differences in intake were found between the 'low-short' and 'high-short' conditions (P=1.0) or between the 'low-short' and 'low-long' conditions (P=1.0). We checked whether low pleasantness ratings for the soup influenced the outcome of the study, by removing the data with pleasantness scores <45 from the dataset. The removal of these data did not change the *ad libitum* intake outcome of the present study.

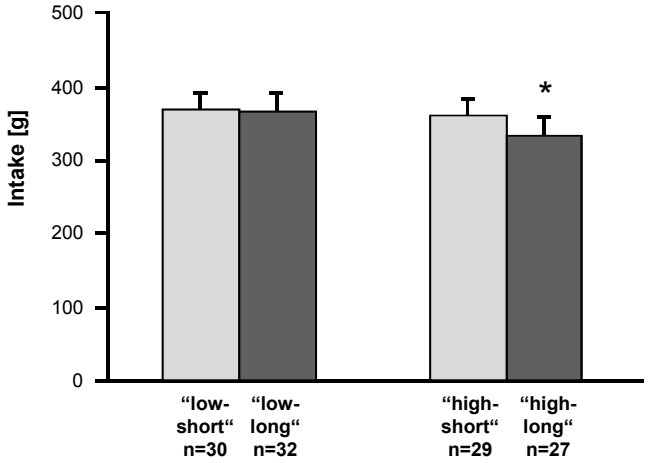


Figure 4.3 *Ad libitum* intake during all the test conditions. Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different from those of the other three conditions (P<0.05).

Appetite and pleasantness ratings

There were no differences in appetite ratings between the test conditions at baseline (t=0; all P>0.05). During *ad libitum* intake, the appetite ratings showed, as expected, a decrease in hunger, desire-to-eat, appetite for something sweet and appetite for something savoury, while fullness and satiation increased (Table 4.1; all P<0.001). Appetite for something savoury decreased more than that for something sweet (P<0.001). Change scores were calculated by subtracting the initial ratings from ratings after 12 minutes of consumption, which equals 240 g of soup intake due to the constant eating rate of 10 g/30 s. After 12 minutes, the dataset contained data from two participants in one condition, three participants in two conditions, twenty-six participants in three conditions and three participants in four test conditions. The change scores of appetite ratings were not affected by exposure time, concentration, or the interaction between exposure time and concentration, measured after 240 g intake (all P>0.05) and just after meal consumption (all P>0.05). The mean maximum pleasantness scores of the four soups were 70 ± 17 for the 'low-short' condition, 69 ± 19 for the 'low-long' condition, 60 ± 19 for the 'high-short' condition and 65 ± 20 for the 'high-long' condition. Soups with a high concentration were rated as more pleasant than those with a low concentration ($F_{1,74}$=4.61; P=0.035).

Table 4.1 Mean values and their standard errors for the initial appetite and pleasantness scores per test condition, measured by using 100 mm VAS and changes in appetite and pleasantness after 12 min soup consumption.

	Hunger	Satiation	Fullness	Desire-to-eat	Appetite for something sweet	Appetite for something savoury	Thirst	Pleasantness
	Initial scores *							
'low-short' (n=30)	68 ± 3.8	22 ± 3.4	19 ± 3.1	73 ± 3.5	55 ± 4.6	66 ± 3.4	68 ± 3.7	56 ± 3.5
'low-long' (n=32)	65 ± 3.7	25±3.3	22±3.0	73±3.3	51±4.4	64±3.2	64±3.5	63±3.2
'high-short' (n=29)	68 ± 3.7	23±3.4	23±3.1	74±3.4	58±4.6	65±3.3	67±3.6	58±3.4
'high-long' (n=27)	65±3.8	29±3.4	22±3.2	73±3.5	58±4.7	66±3.5	64±3.8	63±3.6
	Change after 12 minutes †							
'low-short' (n=27)	-27±3.9	28±4.2	30±4.2	-26±4.0	-10±4.1	-25±4.5	-15±3.6	-17±2.7
'low-long' (n=26)	-24±3.8	26±4.0	31±4.0	-27±3.9	-10±3.9	-21±4.3	-13±3.5	- 9±2.4
'high-short' (n=24)	-28±3.9	31±4.2	32±4.1	-28±4.0	-13±4.0	-25±4.4	-11±3.7	-15±2.7
'high-long' (n=21)	-26±4.0	24±4.5	33±4.4	-27±4.2	-12±4.3	-25±4.6	-15±3.9	-15±3.0

* Initial scores are means ± SE corrected for order, total number of observations=118

† Change scores (initial score - score after 12 min) are means corrected for order and initial scores ± SE, total number of observations=98

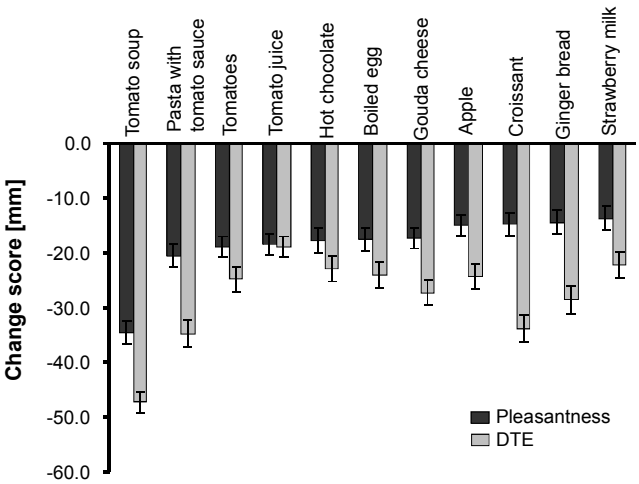


Figure 4.4 Mean change in pleasantness ± SE and DTE ± SE per food, rated on 100 mm VAS, averaged over all test conditions.

Sensory-specific satiation

The pleasantness and DTE of the tomato soup decreased more during food intake than the pleasantness and DTE of the 7 non-tomato products ($F_{1,902}$=74; P<0.001 and $F_{1,902}$=79; P<0.001 respectively; Figure 4.4), which is defined as SSS. The ratings of the 3 tomato products excluding the tomato soup decreased more than the ratings of the 7 non-tomato products using pleasantness ratings ($F_{1,1138}$=7.8; P=0.006), but not using DTE ratings (P=0.93). Ratings of the savoury products decreased more than of the sweet products using pleasantness ratings ($F_{1,1138}$=11; P=0.001), but not using DTE ratings (P=0.73).

Discussion

The present study shows that the amount of odour exposure affects *ad libitum* intake. Since all other sensory factors that can influence food intake were standardised, the differences in *ad libitum* intake that were found in the present study should be attributed to changes in the odour release profile alone. Taste and mouthfeel were the same in all the conditions, because the participants received the same soup base in the mouth. Moreover, eating rate, bite size and time that the product stayed in the mouth were kept the same by using a computerised system with a peristaltic pump and auditory beeps.

A 9% lower food intake was observed when the participants were exposed to the 'high-long' condition with 18 s exposure time and high concentration than when exposed to the other three conditions (Figure 4.3). These results indicate that *ad libitum* food intake depends on a combination of both odour exposure time and odour concentration. An effect of exposure time on food intake was found only at high concentrations. The two odour concentrations used in the present experiment centred on the release concentrations observed during regular tomato soup consumption. The total odour stimulation during the 'low' conditions may have been too small to allow odour exposure time to exert its effects on food intake. Furthermore, odour concentration affected food intake only when the exposure time was long (18 s). Similarly, the total odour stimulation during the 3 s of odour exposure may have been too little to demonstrate an effect of odour concentration on food intake. Possibly, the total amount of odour volatiles is more important for the development of sensory satiation and the subsequent lower food intake, than the separate factors odour concentration and odour exposure time.

The 9% decrease in food intake due to an increase in exposure time that was found in the present study is in line with the results of previous studies.[52, 201] These studies have reported decreases in food intake between 9 and 20% after an increase in sensory exposure time. Kissileff *et al.*[201] showed that participants consumed 20% less yogurt shake

when the eating rate was 70 g/min than when it was 140 g/min. Lowering the eating rate increases the sensory exposure time per bite. Zijlstra *et al.*[52] found a decrease of 9 to 18% in the *ad libitum* intake of chocolate custard when the sensory exposure time was increased from 3 to 9 s. The decrease in *ad libitum* intake was 9% when the bite size was large (15 g) and 18% when it was small (5 g). Similar to that observed in the set-up of the present study, the participants consumed the food through a tube that was connected to a pump, while beeps signalled when to swallow, controlling the time the food is in the mouth. Ruijschop *et al.*[123] found no differences in *ad libitum* intake. We assume that they used a measure that was less sensitive than the one used in the present study. Ruijschop *et al.*[123] measured *ad libitum* intake 10 minutes after the preload with the retronasal odour delivery, whereas we measured *ad libitum* intake during the retronasal odour delivery. Also in a more 'natural' setting, people consume less when foods need longer processing in the mouth.[223] For example, the *ad libitum* intake of liquids is greater than the *ad libitum* intake of solid foods. In all the studies mentioned above, taste and mouthfeel may have contributed to the effect of sensory exposure time on food intake, while in the present study, the effects resulted from differences in odour alone.

In contrast to our expectations, we did not find any differences in subjective appetite and satiation ratings between conditions. Ruijschop *et al.*[123] found an increase in subjective satiation after 8 sips (equal to 8 min) of 43 s, compared with 8 sips of 14 s. They used a technique similar to the one used in the present study: a strawberry odour was delivered retronasally after each sip from a sweetened milk drink. Rolls *et al.*[227] and Zijlstra *et al.*[228] also found an increase in subjective fullness when the sensory exposure time to a fixed preload was longer due to, respectively, air incorporation or increase in viscosity. Although subjective appetite ratings have been shown to predict food intake,[27] some studies, including the present study, have reported no effect on appetite ratings even though an effect on food intake was found.[53, 54, 138, 200, 205] In most of these studies, however, the appetite ratings were recorded after an *ad libitum* intake, while the results of appetite ratings are more comparable with each other after consumption of a fixed amount of food. We measured the appetite ratings after consumption of a fixed amount of soup, but found no effect of odour release profile on appetite ratings. In two studies, Zijlstra *et al.* investigated the effect of consuming foods with different viscosities on fullness after a fixed amount of food[228] and on *ad libitum* intake.[138] The difference in fullness was small (8 mm on 100 mm VAS), while the difference in intake was large (30%). If a 30% difference in intake is accompanied with only small changes in appetite ratings, then no difference in appetite ratings can be expected with a 9% difference in intake, as was found in the present study.

The rated intensity of the soups in the four conditions did not differ between the conditions. This may be caused by a dominant role of taste in flavour intensity; taste intensity was the same in all the conditions. Furthermore, the washout time of at least 5 d in between sessions made it impossible for the participants to compare the four soups used in the present study against each other. They were probably compared against the prototypes of well-known soups, making it more difficult to detect small differences. During the pre-tests, the participants were exposed to the conditions one after the other with a pause of circa 10 s. Possibly, the perceptual differences in intensity were emphasised by a contrast effect.[229]

As expected, eating the tomato soup decreased pleasantness and DTE ratings more for tomato soup than for non-tomato products, named SSS. Additionally, the contribution of odour in the total SSS was investigated, because literature shows that SSS transfers to foods with similar properties such as taste, flavour and texture.[11, 58, 59, 230] The present results show a larger decrease in pleasantness for the tomato products, excluding tomato soup, than for the non-tomato products, which confirms the importance of odour in the development of SSS. However, the DTE ratings did not show such effect.

Furthermore, the participants reported that they had no idea as to how much they had consumed. In the experimental set-up used in the present study, they were not able to see how much they had eaten during *ad libitum* food intake, because they received the soup base via a tube in the mouth. We believe that this is an advantage when studying *ad libitum* intake, because visual cues play an important role in the development of satiation and the selection of portion sizes.[231, 232] We observed an increase in pleasantness and food intake over the sessions, which was largest between the first and the second session. An increase in pleasantness has also been observed in other studies when the participants were unfamiliar with the stimuli.[233, 234] Some participants told us that the soup was somewhat odd, although they believed that they had consumed tomato soup. Probably, the participants of the present study had to get accustomed to either the experimental setting or the soup and the odour. The participants received a retronasal tube in their nose, felt air blowing into their nose, ate from a tube and swallowed when they heard a beep. Therefore, we considered the first session as a training session, resulting in an incomplete design. It is unlikely that the outcomes of the present analysis are artifacts of this incomplete design. The missing values were from randomly chosen conditions and random effects for participants corrected for differences between the participants in the statistical model. Furthermore, we used 1Hz odour pulses differing in duration to adjust odour concentration. In this way, the depletion of odour volatiles was the same in all the conditions. These pulses can be measured with atmospheric-pressure chemical ionisation–

mass spectrometry, as can be seen in the profile 'low-long' in Figure 4.2A, but were perceptually not noticed by the participants during pre-tests. After leaving the outlet of the olfactometer, the odour volatiles travelled for 20 cm through the retronasal tubes before arriving to the nose of the participants. The odour pulses blended into a continuous percept that had an intensity proportional to the average odour concentration.

In the present study, all factors that may influence food intake were standardised as much as possible. Under normal circumstances, the physical properties of foods affect the extent of retronasal odour release during consumption.[213-217] Designing food products that release a large quantity of retronasal odour may contribute to a decrease in food intake, but other factors should also be taken into account. In daily life, many factors other than odour influence food intake. Possibly, small effects of odour on food intake are overruled by major factors such as food palatability.

We hypothesised that an increase in both odour concentration and odour exposure time increases SSS, which in turn increases subjective satiation and decreases *ad libitum* food intake. In line with our hypothesis, an increase in odour concentration and odour exposure time decreased food intake by 9%. The subjective appetite ratings were not affected. Overall, we conclude that it is likely that both odour concentration and odour exposure time play a role in the development of satiation. Possibly, the inconsistency of the data on food intake and subjective appetite ratings reflects the small effect size.

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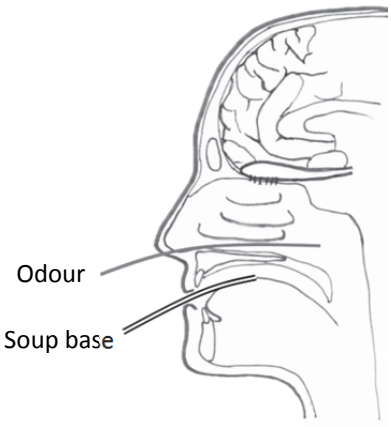
Chapter 4B

Retronasal odour exposure



The influence of cream odour and viscosity on satiation

Submitted for publication. Mariëlle Ramaekers, Pieternel Luning, Rianne Ruijschop, Catriona Lakemond, Martinus van Boekel

Abstract

The sensory characteristics of foods are associated with the metabolic consequences of eating those foods. This learned satiation is believed to guide food intake. It was hypothesised that the addition of cream odour to and an increase in the viscosity of soup both lead to an increase in satiation and a decrease in food intake, because they may both be associated with an increase in energy density. In a 2x2 randomised cross-over study, 42 non-restrained healthy women (18-45 y, 18.5–25 kg/m2) consumed tomato soups differing in cream odour and viscosity. The odour was delivered directly into the nasal cavity via a retronasal tube that was connected to an olfactometer. A tomato soup odour was given in the no-cream condition, whereas cream odour was added in the cream condition. The soups differed in viscosity (liquid, semi-liquid), but were similar in macronutrient composition and energy density. Results showed that addition of cream odour enhanced reported satiation between 7 and 13 min after the start of the soup consumption (P=0.01). Neither cream odour nor viscosity affected *ad libitum* intake. These results may suggest that retronasal odour exposure affect satiation only during the first 10 to 15 minutes of a meal.

Keywords: Ad libitum food intake, Energy density, Odour

Introduction

Nowadays, the prevalence of welfare diseases such as obesity increases rapidly due to our changing lifestyle and eating behaviour.[1] Insight into the determinants of food intake regulation may help in the prevention of overconsumption. As one of those determinants, sensory processes play a role in eating behaviour prior to and during food intake and influence satiation. Satiation refers to the processes that bring a meal to an end.[26] It has been suggested that retronasal olfaction, i.e. odours perceived via the mouth, is linked with satiation.[119, 235] Although the effect sizes are modest, longer and stronger exposure to retronasally administered odours during food intake appears to increase rated satiation and decrease food intake.[119, 235] In the present study, the relation between odour and satiation was explored further.

During daily encounters with foods, humans learned the satiating capacity of foods by linking the sensory characteristics with the nutritional value of those foods.[236] This learned satiation is found to be rather stable in adults[237, 238] and is believed to guide food choices and food intake.[153, 158] Perhaps, this learned satiation can be used to alter satiation, for example by adding an odour that acts as a cue for energy dense foods.[239] The brain may contain fat-sensitive neurons that respond specifically to odours that are associated with fat, e.g. cream odour.[240] In animal studies, it was found that olfaction is needed to develop a preference for many high-fat foods.[241-244] Furthermore, the presence of fat-soluble odour compounds may cognitively influence the estimated amount of energy intake. The expected amount of ingested energy affects the feeling of fullness and the amount of food intake.[231, 245, 246] For example, visible fat in our food increases satiation compared with invisible fat.[231] The many fat-soluble odour compounds in dietary fat may be associated with fat[247] and their presence may be indicative of fat content.[188] Therefore, we suggest that sensing fat by smelling cream may also affect satiation. Adding cream odour may consciously or unconsciously increase the expected energy that is provided by the food, may affect feelings of satiation and maybe even affect food intake.

Further, the most important cues for fat content of foods probably originate from texture properties. Viscosity and creaminess are important attributes of fatty mouthfeel[248, 249] and cues for energy density and may therefore affect satiation. Studies indeed demonstrated that an increase in viscosity increased the expected satiation.[250, 251] Breast fed babies may already learn that there is a positive relationship between viscosity and fat content.[252] Additionally, cross-modal interactions between the cream odour and viscosity may influence possible effects on satiation. Cross-modal interactions are the enhancement of sensory signals when congruent stimuli from different sensory modalities are being

perceived at the same time.[85, 114, 116] For example, adding vanilla odour enhanced perceived sweetness[253] and adding cream odour to custard increased perceived thickness.[117] In the present study, cross-modal odour-texture interactions may strengthen the combined effect of cream odour and viscosity on satiation.

The aim of the study was to investigate if cream odour or viscosity affects satiation, taking into account the multimodal interaction between odour and viscosity. It was hypothesised that the addition of cream odour to and an increase in the viscosity of soup both lead to an increase in satiation and a decrease in food intake, because they may both be associated with an increase in energy density.

Participants and methods

Experimental design

We used a randomised 2 x 2 within-subject design, investigating the effects of odour type and viscosity, resulting in four conditions named no-cream/liquid, no-cream/semi-liquid, cream/liquid and cream/semi-liquid. All participants attended the test location on five separate days, with at least 5 days in between test days. The first session was used to familiarize participants with the test set-up, the procedure and the tomato soup. The actual data were gathered during the subsequent four test days. The eating rate was kept constant at 24 g/min in all conditions.

Participants

We recruited healthy women, aged 18-40 y, BMI 18.5-25 kg/m^2. Potential participants filled out an inclusion questionnaire. Exclusion criteria were: dislike tomato soup (score <5 on a 9 points scale), restrained eater (Dutch Eating Behavior Questionnaire score >2.79[159]), energy restricted diet during the past two months, change in body weight >5 kg during the past year, stomach or bowel diseases, diabetes, thyroid disease or any other endocrine disorder, having difficulties with swallowing/eating, hypersensitivity for any of the ingredients of the soup, smoking, pregnant or breastfeeding during the past half year or lack of appetite. In total 42 participants aged 21.8 ± 4.4 y, with a mean BMI of 21.2 ± 1.8 kg/m^2 completed the study and received financial compensation. Participants were kept naïve to the exact purpose of the study and were informed that this study was about the investigation of the mechanism of smell and taste. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethical Committee of Wageningen University. All participants signed an informed consent form.

Soup base and odour delivery

The odour was delivered separately from the consumed soup base, because a change in viscosity possibly influences the odour release.[254, 255, 256] The tomato soup odour consisted of 6 g of tomato flavour (15.04.0166, IFF, Hilversum, the Netherlands), 8 g of yeast extract (55061TT, Givaudan, Naarden, the Netherlands) and 1 g of Italian herb flavour (RW-896511-6, Givaudan, Naarden, the Netherlands) dissolved in 85 g water. The cream odour contained 10 g of cream flavour (QL97645, Givaudan, Naarden, the Netherlands) dissolved in 90 g of warm water. Ten ml of each odour was added to two separate odour vessels of a computer controlled four-channel olfactometer based on air-dilution olfactometry (OM; Burghart, Wedel, Germany). Odour pulses were generated by switching between odourised air and non-odourised air, while keeping the total flow rate at 9 L/min. In the no-cream condition 0.5 L/min tomato soup odourised air was mixed with 8.5 L/min clean moisturized air. In the cream condition 0.5 L/min tomato soup odourised air, 0.5 L/min cream odourised air and 8.0 L/min clean moisturized air were mixed. The odour solutions were refreshed every 100 s to limit depletion of volatiles. A medically trained person inserted a 'retronasal tube' (silicon suction catheter with a total length of 20 cm, CH 10, D-Care B.V., Houten, The Netherlands) for 8 cm into the noses of the participants, with the outlet positioned at the retropharynx of the soft palate. The retronasal tube was subsequently connected to the olfactometer for retronasal odour delivery. Participants could breathe normally.

The soup base contained 132 kJ/100g and consisted of 561 g cooked water, 75 g Sugocasa tomato sauce (Grand' Italia, the Netherlands), 8 g Maggie Bouillon (Nestle, The Netherlands), 2 g Tomato Crème Cup á Soup (Unilever, the Netherlands), 2 g cream (coffee cream, Campina, the Netherlands), 28 g modified starch 'Honig allesbinder' (Heinz, the Netherlands) and 8.5 g of starch (liquid soup base: Perfectamyl A3108; semi-liquid soup base: Farinex VA40). We used different types of starch to develop identical products differing in viscosity only (viscosities are shown in Figure 4.5). Batches of 600 g soup base were kept at 60 °C using a water bath, but the soup was consumed at a temperature of circa 55 °C. The participants received the soup base into their mouth via a silicon tube (diameter 4.8 mm, Rubber B.V., Hilversum, The Netherlands), using an electric peristaltic pump (Watson-Marlow, Bredel, USA). The silicon tube was insulated with aluminium foil to prevent heat losses. Each time a sip of soup was swallowed, the tomato soup and the cream odours were delivered retronasally during 12 s. This resulted in a realistic impression of tomato soup.

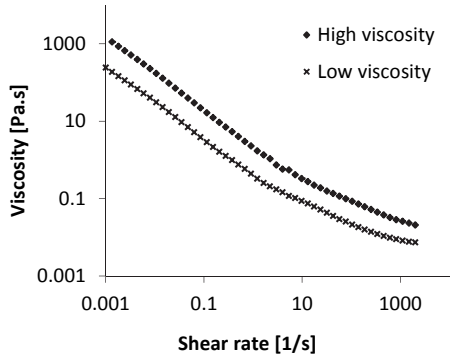


Figure 4.5 Viscosities of soup bases with high and low viscosity, measured with a rheometer (ARES, Rheometric Scientific, USA) at 55 °C and shear rates increasing from 0.001 to 1000 1/s in 8 min.

Pilot

Prior to the real experiment, several pilot studies were done to find congruent combinations for soup base and odours. During the last two pilot days, we collected data on the four final conditions. Five young, healthy women rated expected satiation, thickness and presumed energy density, during soup consumption, on 100 mm visual analogue scales (not at all – very much). Each person evaluated three out of the four conditions on the same day instead of four conditions, to avoid ratings when completely satiated. Ratings were repeated after intake of 20, 60 and 100 g of soup and averaged per person and per condition for data-analysis.

Procedure

Participants were instructed to consume a normal amount of food and drinks until two hours before the start of the experiment. After this time only water and weak tea were allowed. The other four test days, the participants were asked to consume the same breakfast as on the first day to standardise their individual state of satiety. Participants were tested between 10.40 h – 14:20 h.

After arrival, the participants first filled out the 'appetite questionnaire' including questions about hunger, satiation, fullness, desire-to-eat, desire-to-eat something savoury, desire-to-eat something sweet and thirst, measured by using 100 mm visual analogue scales (not at all - very much). After that, the retronasal tube was inserted into the nose and connected to the olfactometer. Then, the participants received one sip (10 ± 0.04 g) of soup base every 25 s, which was around three times slower than during normal soup consumption.[223] Beeps notified them when a sip of soup would come into their mouth, when to swallow and when to complete questionnaires. The olfactometer, the peristaltic pump, the beeps and an instruction screen were synchronised using

previously described software.[117] After three sips, the participants rated pleasantness of the soup (not at all - very much), overall flavour intensity (not intense - very much intense), saltiness, sweetness, sourness and bitterness (not at all - very much) on 100 mm visual analogue scales. Furthermore, the participants repeated the appetite questionnaire plus the question on pleasantness of the soup after 80, 160, 240, 320, 400, 500, 600, 700, 800 and 1000 g of soup intake and directly after finishing eating. The participants were requested to consume tomato soup until they felt pleasantly full. The retronasal tube stayed in the nose for at least 25 minutes, even if the participant already finished eating.

Data Analysis

Data are presented as mean ± SD, unless stated otherwise. Data were analysed using SAS (version 9.2; SAS Institute Inc., Cary, NC, USA). Results with $P<0.05$ were considered significantly different. The first session was set-up for training and was therefore excluded from the analysis.

The number of sips was counted to determine the *ad libitum* soup intake. The effects of cream odour, viscosity and their interaction on *ad libitum* soup intake, appetite ratings, pleasantness of the soup ratings, expected satiation (pilot), thickness and presumed energy density were tested using mixed linear models with participant as random variable. For *ad libitum* intake, pleasantness of the soup was included as covariate. Covariates in all other analyses were: ratings at baseline (t=0), time and session.

The number of appetite and pleasantness questionnaires that participants filled out during soup consumption varied among the participants and test conditions, because they stopped eating at different moments. At baseline (t=0), all 42 participants filled out the questionnaire in all four conditions. There were 31 participants left with complete questionnaires in all conditions after 320 g of soup intake (13 min) and 23 participants were left after 400 g (17 min). The data of the appetite and pleasantness questionnaire were analysed after 320 g, as a trade-off between including as many as possible data points and including as many as possible participants with complete sets of questionnaires. *Post-hoc* tests were used to test for differences between conditions at separate time points. Additionally, the data from 23 participants who ate at least 400 g of soup in all four conditions were visually presented, in order to include one more data point. Data from four sessions were removed: three participants reported on one test day a lack of appetite due to illness, heavy cold or hay fever and one participant did not obey to the instructions prior to the test.

Results

The four odour conditions

Semi-liquid soup was rated higher on expected satiation, on perceived thickness and on expected energy density (Table 4.2). Although not significant, adding cream odour tended to increase expected satiation and thickness, in combination with a semi-liquid soup, but not with a liquid soup.

Table 4.2 Mean values for expected satiation, thickness and expected energy density rated by five young, healthy women during a pilot study, on 100 mm visual analogue scales (not at all – very much) prior to the real experiment.

	liquid				semi-liquid				P-values		
	no-cream (n=4)		cream (n=4)		no-cream (n=3)		Cream (n=4)		cream	viscosity	interaction
	mean	SD	mean	SD	mean	SD	mean	SD			
Expected satiation	59	6	56	10	63	7	78	11	0.320	**0.0146***	0.117
Thickness	51	5	48	12	58	11	70	16	0.630	**0.0059***	0.510
Energy density	45	10	43	21	67	13	68	22	0.990	**0.0162***	0.690

* P-value <0.05

Ad libitum soup intake

Cream odour ($F_{1,118}$=0.12; P=0.73), viscosity ($F_{1,118}$=0.72; P=0.40) and the interaction between cream odour and viscosity ($F_{1,118}$=0.45; P=0.51) did not affect *ad libitum* soup intake. Mean intake was 501 ± 197 g in condition no-cream/liquid, 506 ± 199 g in no-cream/semi-liquid, 516 ± 205 g in cream/liquid and 498 ± 179 g in cream/semi-liquid. *Ad libitum* intake correlated with pleasantness (ρ=0.27; P<0.001), but not with session (ρ=0.07; P=0.36).

Table 4.3 Hunger, desire-to-eat, satiation and fullness ratings averaged over time, and per time point, measured by using 100 mm VAS (not at all – very much) of 31 participants who consumed at least 320 g soup in all four conditions. P-value for the effects of cream, viscosity and their interaction are given and when (borderline) significant the results of post-hoc tests.

		liquid				semi-liquid				P-values		
Time	Intake	no-cream		cream		no-cream		Cream		cream	viscosity	interaction
[min]	[g]	mean	SD	mean	SD	mean	SD	mean	SD			
Hunger		52	25	51	21	54	21	51	21	0.061	0.72	0.98
0	0	60	24	63	25	65	22	64	20	0.850		
3	80	59	24	61	21	62	18	61	16	0.875		
7	160	58	24	55	19	58	21	55	19	0.094		
10	240	50	24	48	20	53	20	48	21	0.053		
13	320	42	24	42	23	44	21	42	23	0.447		
Desire-to-eat		60	22	61	20	61	21	58	22	0.078	0.19	0.39
0	0	66	24	70	19	70	20	69	21	0.691		
3	80	67	20	69	19	67	18	66	20	0.825		
7	160	64	21	63	19	65	20	60	20	**0.049***		
10	240	57	22	59	18	59	21	57	22	0.480		
13	320	51	25	52	21	54	22	50	23	0.114		
Satiation		37	25	39	23	37	21	39	22	**0.009***	0.90	0.79
0	0	30	25	25	21	27	19	26	17	0.575		
3	80	29	24	28	21	26	17	28	17	0.449		
7	160	32	23	35	24	33	19	36	19	**0.030***		
10	240	39	25	43	21	40	22	43	21	**0.032***		
13	320	48	25	48	23	48	21	52	24	0.080		
Fullness		37	25	40	23	36	20	38	23	**<0.001***	0.58	0.90
0	0	27	24	21	16	24	16	19	15	0.227		
3	80	29	23	28	20	25	15	24	15	0.287		
7	160	33	24	36	22	31	17	35	20	**0.008***		
10	240	38	25	44	20	38	21	43	23	**<0.001***		
13	320	47	27	50	23	47	21	51	25	**0.008***		

*** denotes significance at P<0.05**

Appetite and satiation

After 320 g of soup consumption, only 31 out 42 filled out the hunger and appetite questionnaire in all four conditions. Therefore, statistical analysis was performed on data of 31 participants, which was a trade-off between including as many data points as possible and including as many participants as possible. All appetite and satiation ratings, except thirst, changed over time during soup consumption (all P<0.001). Adding cream odour affected hunger, fullness, satiation and desire-to-eat ratings between circa 7 and 13 minutes in a consistent way, although not all differences were significant (Table 4.3; n=31). Fullness and satiation increased more over time in the cream conditions than in the no-cream conditions ($F_{1,545}$=15.6; P<0.001 and $F_{1,545}$=7.9; P=0.009, respectively). Hunger and desire-to-eat decreased more in the cream conditions than in the no-cream conditions, but these differences were borderline significant ($F_{1,545}$=3.8; P=0.061 and $F_{1,545}$=3.3; P=0.078, respectively). Post-hoc comparisons revealed that the differences in fullness, satiation and desire-to-eat between the cream and no-cream conditions became significant seven minutes after the start of the soup consumption and lasted until 10 - 13 min. The effects of cream odour on hunger and fullness are shown in Figure 4.6 for visualisation of the appearance and disappearance of effects (n=23). Furthermore, adding cream odour had no effect on the desire-to-eat sweet products, the desire-to-eat savoury products or thirst (all P>0.20; n=31). Viscosity and the interaction between cream and viscosity had no effect on any of the appetite ratings (all P>0.05), except that the desire-to-eat sweet products was higher during intake of the liquid soup, than during intake of the semi-liquid soup ($F_{1,545}$=4.6; P=0.039).

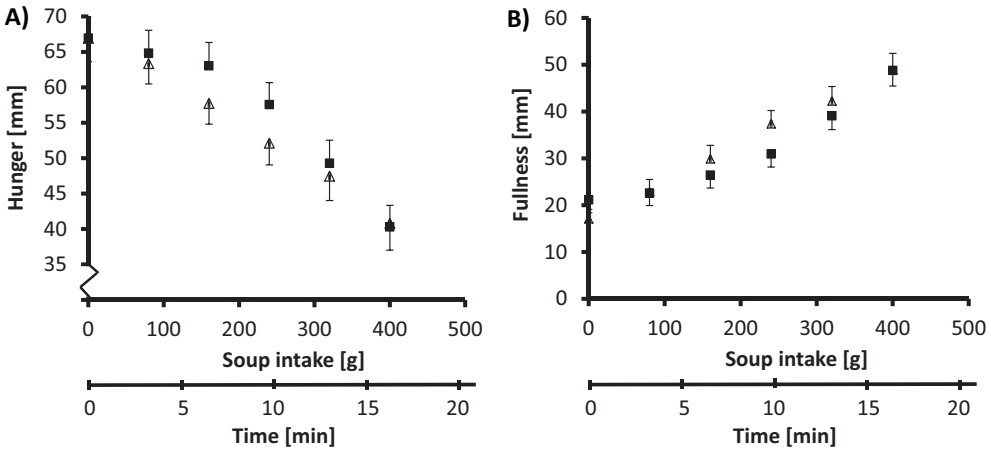


Figure 4.6 Mean ± SEM of the hunger (**A**) and fullness (**B**) scores of 23 participants in the no-cream condition (■) and the cream condition (Δ) as a function of soup intake in grams and as a function of time in minutes, measured by using 100 mm VAS.

Pleasantness

Figure 4.7 shows the development of pleasantness in all conditions over time. Over all time points, the soup in no-cream/semi-liquid was liked over the soup in the other three conditions (all P<0.01).

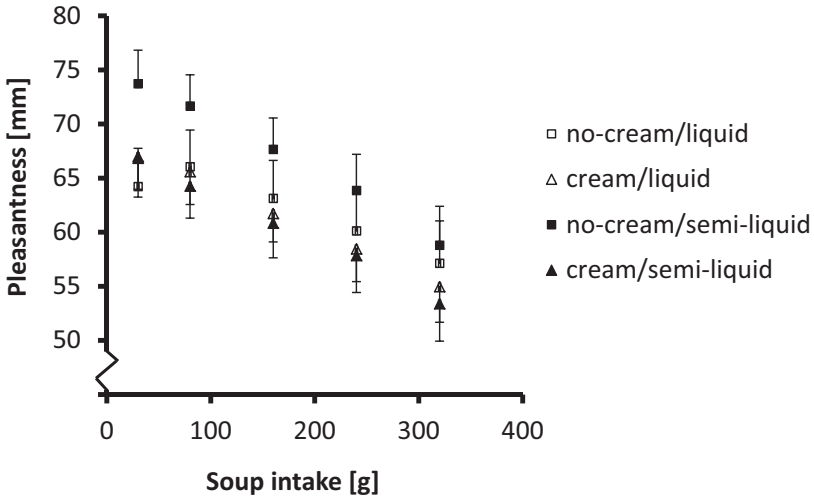


Figure 4.7 Mean ± SEM of the pleasantness scores of 31 participants in all conditions as a function of soup intake in grams and as a function of time in minutes, measured by using 100 mm VAS (not at all – very much).

Taste intensity

Adding cream odour decreased saltiness (Table 4.4; P=0.0153) and had no significant effect on sweetness, sourness or bitterness (all P>0.05). Viscosity and the interaction between cream and viscosity had no effect on taste intensity (all P>0.05).

Table 4.4 Saltiness, sweetness, sourness, bitterness and total flavour intensity rated after 30 g of soup intake (n=42).

	liquid				semi-liquid				P-values		
	no-cream		cream		no-cream		cream		cream	viscosity	interaction
	mean	SD	mean	SD	mean	SD	mean	SD			
Sweet	44	22	45	20	43	20	44	19	0.880	0.750	0.980
Salt	58	18	51	19	55	17	52	21	**0.015***	0.910	0.290
Sour	30	21	24	20	24	20	23	17	0.091	0.270	0.460
Bitter	19	16	19	18	16	18	16	17	0.970	0.200	0.840

*** denotes significance at P<0.05**

Discussion

The aim of the study was to investigate if adding cream odour or differences in viscosity affect satiation, taking into account the multimodal interaction between odour and viscosity. Ratings of fullness and satiation were higher (significant) and hunger and desire-to-eat appeared to be lower (borderline significant) in the cream than in the no-cream conditions between circa 7 and 13 minutes after the start of the tomato soup intake. The simultaneous appearances and disappearances of the differences in the appetite and satiation ratings (Table 4.3; Figure 4.6) may possibly suggest that different processes were involved at different times, although the differences were small and no correction was made for multiple comparisons. No differences were found in *ad libitum* intake.

In the cream conditions, participants sensed more cream than was actually present in the soup. The effects of cream odour on satiation peaked around 7 minutes after which the effect disappeared after 13 minutes (Figure 4.6). Perhaps the sensory perception of the cream odour increased feelings of satiation at first. However, later the energy content of the soup might have been detected in the gastro-intestinal tract (GI tract), overruling the sensory information, and diminishing differences between odour conditions. In rats with intragastric infusion via catheters, it took six minutes before they preferred a saccharine solution to water.[257] Also mice that could not taste sweet still preferred sucrose over water after ten minutes,[258] probably by detection of sucrose in the GI tract. In humans, the gastric emptying of some soups takes around 14 minutes,[259] after which the soup is transported to the small intestine. Fat in the small intestine slows down gastric emptying[260, 261] and affects hormone release in the gastro-intestinal tract, both influencing appetite and food intake.[262-264] De Araujo *et al.*[258] suggested that calorie-rich nutrients directly influence brain reward circuits that control food intake. Therefore, it may be assumed that once the processes in the GI tract responded to the nutrients in the soup, they might have overruled the sensory information. If these suggestions hold true, then it could explain why no differences in food intake were found in the present study, because the moment of meal termination was on average 20 minutes and was much later than the thirteen minutes at which metabolic processes possibly joined. It may be hypothesised that the addition of cream odour increased satiation at first, although the differences were small, but that processes via the GI tract overruled the sensory information after 13 minutes.

If cognitive expectations about energy density would be decisive for feelings of satiation, we would expect a larger effect of viscosity on satiation than of cream odour, because the effect of viscosity on the expected satiation, thickness and expected energy density was much larger than the effect of cream odour (Table 4.2).[250] However, cream odour influenced the satiation ratings while viscosity did not. Cream odour may be associated specifically with fat, while viscosity was not. Cecil *et al.*[56] found that sensory information about fat content is important for subsequent appetite and gastro-intestinal responses. Eating high-fat soup suppressed hunger, induced fullness and reduced energy intake, compared with eating iso-caloric high-carbohydrate foods, which demonstrates the satiating effect of fat irrespective of energy density.[56] These differences, however, were not present when the soup was infused intra-gastrically, eliminating sensory exposure.[56] On the other hand, viscosity was associated with energy density (Table 4.2), but this did not result in differences in rated satiation or food intake though. Zijlstra *et al.*[138] also found that viscosity had no significant effect on satiation when the eating rate was fixed with a pump system, similar to the one used in the present study. Rolls and colleagues demonstrated in a series of experiments that some neurons respond to fat, independently of viscosity, whereas other neurons respond to viscosity.[265] This implicates that increase in viscosity without the presence of fat does not increase fat perception, although viscosity is an important attribute in fatty mouthfeel. In a recent study, Frank *et al.*[131] showed that addition of olive extract to yoghurt, without the fat, increased blood flow in the primary taste cortex, implicating that it might be possible to simulate fat-triggered sensations by ingredients that are associated with fat. Perhaps an association to fat or cream by the cream odour was more important for cue-induced satiation than an association with energy density as influenced by the viscosity.

Another possible explanation for the current increase in satiation feelings is odour complexity. Ruijschop *et al.*[122] found that participants felt more satiated when they were exposed to a retronasal strawberry odour that consisted of more compounds, thus was more complex, than when exposed to a single component that is perceived as strawberry. In the present study, adding the cream odour might have been perceived as more complex, increasing satiation feelings. The disappearance of the effect of cream odour over time, may have been caused by adaptation, i.e. inability to perceive differences between odours. The true underlying mechanisms of the observed differences remain to be determined.

The initial pleasantness ratings were acceptable for all soups. The pleasantness of the soup in the no-cream/semi-liquid condition was liked better than in the other three conditions, which may affect satiation feelings and food intake. Possibly, pleasantness mediates satiation feelings,[266] with lower pleasantness resulting in higher satiation ratings. However, no-cream/liquid and cream/liquid were rated as equally pleasant, whereas differences in hunger and satiation were also present between these low viscosity soups. Furthermore, equal pleasantness of stimuli is regarded as important when measuring *ad libitum* intake, because of its strong influence on intake.[21] In the present study, however, we found no difference in *ad libitum* intake, despite differences in pleasantness. Therefore, we assume that the effects on subjective ratings and food intake in the present study were not mediated by pleasantness.

Interestingly, saltiness decreased when the cream odour was added via the retronasal tube. It is generally known that adding cream to food changes taste and flavour intensities.[e.g.267] Real fat in food may obstruct taste perception by e.g. covering taste buds, making them less accessible for tastants. However, in the present study, no cream was actually added to the soup base and the cream odour could not interact with the taste buds, because it was administered directly into the nose. Differences in saltiness are therefore attributed to cross-modal interactions. The brain integrates sensory signals from different modalities into a new percept via cross-modal interactions.[e.g.115, 117]

In conclusion, adding cream odour temporarily affected reported satiation during intake of tomato soup and did not affect *ad libitum* intake, suggesting that retronasal odour exposure affect satiation during the first 10-15 minutes. After that, metabolic processes may overrule the sensory perception. Viscosity did not affect reported satiation or *ad libitum* food intake.

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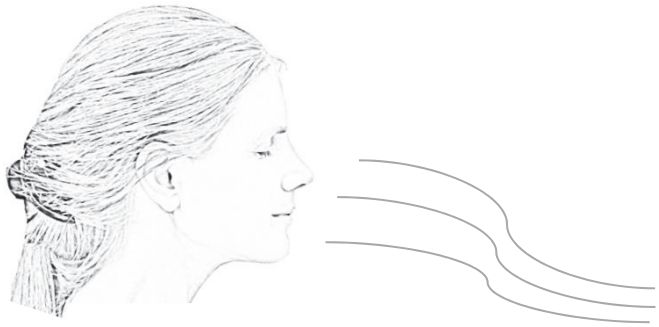
Chapter 5

General discussion



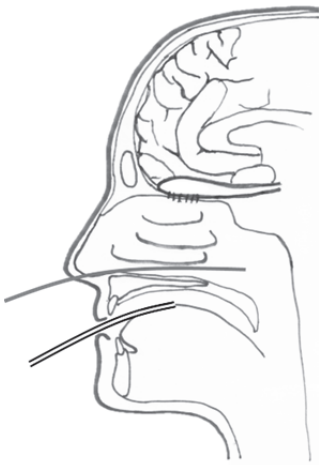


Table 5.1 Overview of main results of this thesis, averaged per variable

	General appetite or satiation VAS[a]	**SSA or SSS** VAS[a]/FPQ[b]	**Change in appetite for *congruent* sweet and savoury foods** VAS[a]/FPQ[b]	**Change in appetite for *incongruent* sweet and savoury foods** VAS[a]/FPQ[b]	**Pleasantness of the odour** VAS[a]	**Food intake** *Ad libitum*	**Actual food choice**
Orthonasal passive							
Exposure time	NS[a] (2A+2B)	NS[ab] (2A+2B)	NS[ab] (2A+2B)	NS[ab] (2A+2B)			
Sweet odours	+4mm[ac] (2A)	+10mm[a]/+30%[b] (2A)	+5mm[a]/+20%[bc] (2A)	-5mm[a]/-21%[bc] (2A)			
Savoury odours	+4mm[ac] (2A)	+15mm[a]/+26%[b] (2A)	+6mm[a]/+31%[bc] (2A)	-5mm[a]/-25%[bc] (2A)			
Bread odour	+6mm[ac] (2A)	+10mm[a]/NA (2A)	NA	+4mm[a]/NA (2A)			
Non-food odours	-2mm[ac] (2A)	NA	NA	NS[a]/NS[c] (2A)			
Orthonasal active							
Exposure time	NS[a] (3A+3B)	NS[a] (3A+3B)	NS[a] (3A+3B)	NS (3A+3B)	-4mm[a] (3B)		
Sweet odours	NS[ac] (3A) NS[ac] (3B)	+12mm[a] (3A) +7mm[a]/+25%[bd] (3B)	+4mm[ac] (3A) NS[ac]/NA[g] (3B)	-6mm[ac] (3A) -4mm[ac]/NA[g] (3B)	NA	NS (3A)	
Savoury odours	NS[ac] (3B)	+7mm[a]/+35%[bd] (3B)	NS[ac]/NA[g] (3B)	-5mm[ac]/NA[g] (3B)	NA		
Switch between sweet and savoury odours	NS[a] (3B)	NA	Adjusted to what is smelled last[ab] (3B)	Adjusted to what is smelled last[ab] (3B)	NS[a] (3B)		Appears to adjust to what is smelled last – no statistics (3B)
Retronasal							
Concentration/ Exposure time	NS[a] (4A)					-9%[e](4A)	
Adding cream odour	-5mm[af] (4B)					NS (4B)	

NS = not significant, NA = not applicable. [a] Measured by using 100mm visual analogue scales (VAS), [b] determined with the food preference questionnaire (FPQ),
[c] compared with the no-odour condition, [d] compared with incongruent odour,
[e] increase in both concentration and exposure time, [f] only between circa 7 and 13 minutes, [g] congruent and incongruent are entangled.
Positive values represent appetizing effects and negative values represent satiating effects. Chapter numbers are given between brackets.

Introduction

The main objective of this thesis was to investigate under which circumstances odours are appetizing or satiating in order to identify factors that influence eating behaviour. Different factors of ortho- and retronasally smelled odours in relation to appetite or satiation were investigated. This general discussion starts with an overview of the main findings. Next, the results will be discussed, followed by a conceptual framework and new hypotheses. After that, the main methodological considerations will be addressed. Finally, the results are put in perspective and it is discussed what the implications are for science and practical food applications. Based on this, suggestions are given for future research.

Main findings

The appetizing and satiating effects of ortho- and retronasally smelled odours were investigated by varying the **odour exposure time**, the **odour concentration** (retronasal only), the **odour type** and by **switching between odour types**. In addition, the orthonasal odours were smelled **passively** in rooms with ambient odours and **actively** by sniffing the contents of a cup.

The main results, averaged per variable, are given in Table 5.1. They show that orthonasal exposure to food odours influenced the appetite for specific foods via a typical pattern: the appetite ratings for the smelled foods increased by +6-20 mm (SSA), the appetite for congruent sweet and savoury foods increased by +5 mm and the appetite for incongruent sweet and savoury foods decreased by -5 mm, measured by using 100 mm VAS (**studies 2A, 2B, 3A and 3B**). The elevated appetite for the smelled foods did not change during a twenty-minute period (**studies 2A, 2B, 3A and 3B**) and did not differ between passive and active smelling (**studies 2A and 3A**). Similar results were found with a computerised food preference questionnaire, in which participants chose repeatedly between pairs of foods (**studies 2A, 2B and 3B**). Results in **study 3B** showed that the appetite for specific products adjusted to the new odour within one minute after a switch between sweet and savoury odours and followed the typical pattern of a categorised appetite response (**study 3B**).

General appetite increased by +4 mm during passive exposure, independent of exposure time (**studies 2A and 2B**). General appetite was not affected by active smelling (**studies 3A and 3B**). Switching between odours had no significant effect on general appetite (**study 3B**). Passively smelled food odours had a larger influence on sensory-specific appetite (+15 mm) than on general appetite (+4 mm; **studies 2A and 2B**). Salivation was not affected by any of the passive odours under study (**studies 2A and 2B**). Interestingly,

odour pleasantness decreased by 4 mm over time during active smelling, whereas the appetite for the smelled food remained elevated (**study 3B**).

An increase in both retronasal odour exposure time and concentration reduced *ad libitum* intake by 9 %, but had no effect on subjective satiation (**study 4A**). Adding cream odour decreased subjective satiation between 4 and 13 minutes after the start of consumption, but did not affect *ad libitum* intake (**study 4B**). A SSS test using photographs demonstrated the contribution of retronasally smelled odour to the development of SSS (**study 4A**). The main results are brought together in Figure 5.1.

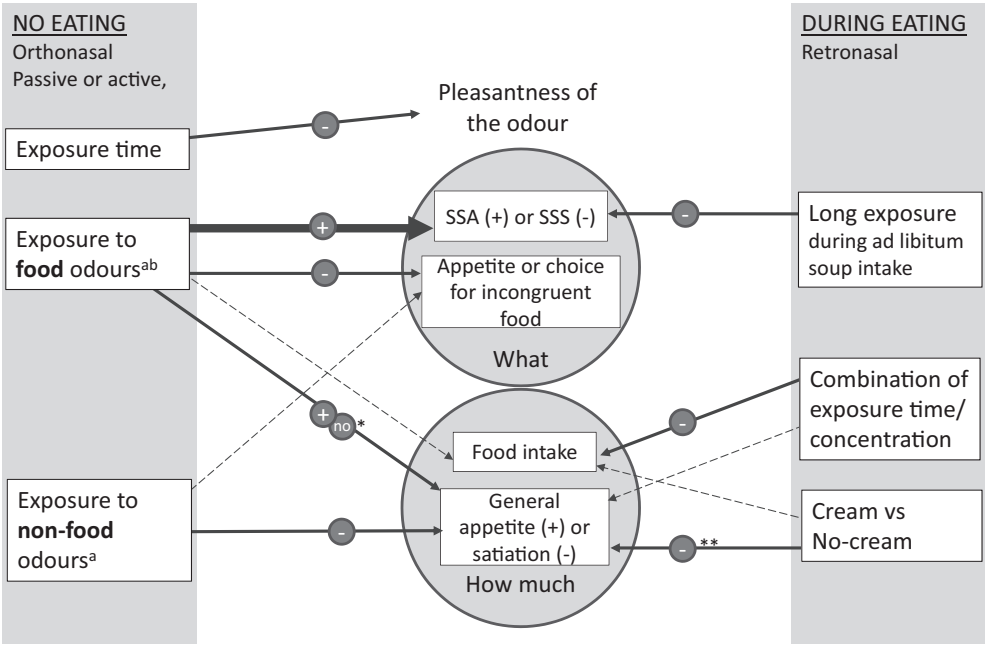


Figure 5.1 Synthesis of the main results of this thesis. The continuous lines represent significant relationships with the strongest one shown as thick line. The plus sign indicates a positive relationship and the minus sign a negative. The dashed lines represent non-significant relationships. All data were generated using a 100 mm VAS. SSA and the choice for incongruent foods were also determined with a food preference questionnaire, which provided similar results as the VAS. [a] Independent of exposure time. [b] Independent of switching between odours. *During passive smelling a significant increase of 4 mm in general appetite was found (study 2) and during active smelling no effect (studies 3A and 3B). **The effect was present between circa 7 – 13 min after the onset of eating. Please find the definitions of SSA and SSS in the glossary.

Discussion and interpretation of the results

The results from this thesis show that exposure to orthonasal food odours increased the appetite for smelled foods and decreased the appetite for incongruent foods (the 'what' in Figure 5.1) and to a minor extent influenced general appetite with no effect on food intake (the 'how much' in Figure 5.1). These effects did not change over a twenty-minute period. Retronasal odours contributed to the development of sensory-specific satiety (what) and influenced general appetite or food intake (how much). As explained in the introduction, SSA, SSS and food preference are considered to measure what is eaten and both subjective appetite ratings and *ad libitum* intake are considered to measure how much is eaten.

Orthonasal exposure (chapters 2 and 3)

In the orthonasal studies, we investigated the effects of exposure time (studies 2A and 2B), active versus passive exposure (study 3A) and switching between odours (study 3B) on the general appetite and the appetite for specific foods. First, we discuss the findings of the categorised response, which was found in all studies. Then, we discuss the effects of the factors that were investigated and finally, hypotheses are formulated based on results that so far are explained by SSA and SSS.

1. Categorised responses

We investigated how different odours influence the appetite for a selected set of sweet, savoury and staple foods, under various circumstances (studies 2A, 2B, 3A and 3B). We showed that sweet and savoury food odours increased the appetite for the smelled foods and other congruent foods. In addition, sweet odours decreased the appetite for savoury foods and savoury odours decreased the appetite for sweet foods. Bread odour increased the appetite for staple and savoury foods and had no effect on sweet foods. Non-food odours did not affect the appetite for sweet, savoury and staple foods. Furthermore, the findings of the food preference questionnaire (FPQ) showed that participants shifted their food preference in 15-25% of the choices in favour of the smelled food.

In this section, we first address the possibility of categorisation that is based on appetite responses. Then we explain the exceptions in the increase in the appetite for congruent foods, followed by hypotheses on odour-nutrient relationships and hypothetical causes for the categorised response. We conclude the section by relating the VAS ratings to the FPQ.

1.1 Categorisation of appetite response

The present results suggest that humans have **separate appetite responses to sweet, savoury, staple and non-food odours**. Blundell *et al.*[21] underscored the importance of the sweet vs savoury dimension in effects of sensory signals on food preference. Ferriday *et al.*[37, 38] also found a categorised appetite response: the sight and smell of pizza elicited an increase in the desire-to-eat (appetite for) savoury foods and a decrease in sweet foods. Fedoroff *et al.*,[36] using cookie and pizza odours, suggested that food odours affect intake only when the food and the odour are similar. Rats also increased their intake of cued foods, but not of uncued foods during cue exposure.[268] Castro *et al.*[187] found ten main odour categories: four related to sweet, i.e. citrus, fruity, vanilla/chocolate/caramel and minty, non related to savoury and five to non-food.[187] This categorisation was based on the perception of the odours. Possibly, the appetizing effects of odours can be categorised in sweet, savoury, staple and non-food and differs from categorisation based on perception as found by Castro *et al.*[187]

1.2 Graded scale in congruency

In all orthonasal studies of this thesis, the appetite for smelled foods increased and the appetite for incongruent foods decreased (studies 2A, 2B, 3A and 3B). However, the appetite for the congruent sweet and savoury foods did not increase in all cases. We observed in study 3B that banana odour did not affect all sweet foods, meat odour not all savoury foods and in study 2A, the chocolate odour not all sweet foods (Table 2.2). Additionally, the increase in the appetite for smelled foods was higher than the increase in the appetite for other congruent sweet and savoury foods (Table 5.1). **Congruency between an odour and a food is probably a graded scale and possibly affects the extent of the appetite response**. Perhaps, the more similar the odour and the food are, the higher the influence of the odour on the appetite for that food. In line with this, results from studies on SSS showed that eating a food to satiety also affected SSS for foods with similar sensory properties as the eaten food.[11, 13, 58, 174] Therefore, it may be proposed that the closer the association of a food cue to a food, the stronger the development of the SSA for that food, with sweet foods evidently incongruent with savoury odours and savoury foods incongruent with sweet odours.

1.3 Odour-nutrient relations

Furthermore, the basis why humans perceive certain odours as sweet and others as savoury or non-food is probably due to **learning.** Foods are associated with their sensory properties and their nutrient composition via post-ingestive feedback of daily exposures (i.e. flavour-nutrient learning).[236] The nutrient compositions of these associated foods differ between each other. In a study investigating fifty different common foods, it was

found that sugar content correlates with sweetness and protein and salt with savouriness,[28, 31, 186] sustaining the suggestion that taste provides information on the nutritional value of foods. Staple foods seem to be a different category and may be associated with non-sweet carbohydrates that take a great part of our daily intake into account.[269] Categorisation into sweet, savoury and staple odours, as found in the present thesis, may help the selection of foods with specific nutrients to facilitate ingestion of a balanced meal. Thus, odours provide information about the availability of foods and may also inform us what kind of foods are present. Odours are closely related to the foods they originate from and therefore **we postulate that odours provide information on the nutrient composition of the food they originate from**.

1.4 Two possible explanations for categorised response

The increase in appetite for smelled foods and decrease in appetite for incongruent foods probably increases the chance of ingesting the smelled food. Interestingly, **no nutrients were yet ingested and therefore this particular shift in preference is not caused by a shift in the need for particular nutrients.** From an evolutionary perspective, ingesting the food that is smelled may be beneficial, because it enhances intake. Although highly hypothetical, **two possible explanations** are proposed. First, the body may prepare itself specifically for the intake and digestion of the smelled food, with its specific macronutrient content and distinct route of digestion, via cephalic phase responses.[42, 43, 48, 193] We hypothesised that it may be less favourable to ingest a food with a very different (macro) nutrient composition, once prepared for the digestion of the cued food. However, results in study 3B show that the categorised response adapts to a new odour within one-minute exposure, which makes it less likely that preparation of the body is the reason of the decrease in the appetite for incongruent foods. **Another possible explanation** for the decrease in the appetite for incongruent foods lies on a cognitive level. The increase in motivation to eat the available food possibly increases the chance for survival, because it helps to store food for scarce times. Simultaneously decreasing the appetite for foods that are not around may strengthen the specific desire for the cued food that is available. These two explanations are highly hypothetical. The underlying reasons for the categorised increases and decreases of specific appetites have not yet been clarified.

1.5 Effect on food preference

Finally, it was found that food odours shifted food preference (measured with FPQ) in favour of the smelled food, which puts the effects that were found on VAS in perspective. However, these data also show that around 80% of the food choices were not affected by odour exposure. Likely, the choice between two foods only shifts when the initial

preferences are similar.[195] Probably, food odours shift food preference, but do not overrule strong initial preferences.

2. Impact of passive vs active, switching and exposure time on general appetite and SSA

In this thesis, it was also studied if active versus passive smelling, odour switching and odour exposure time affected general appetite and the appetite for specific foods.

2.1 Passive vs active smelling (chapters 2 versus 3)

It was hypothesised that active sniffing, rather than passive exposure induces the satiating effects of odours over time. In the studies in studies 2A and 2B, orthonasal odours were offered passively without notification of the participants, whereas in other studies (studies 3A and 3B) odours were offered actively by sniffing the contents of a cup. The results show that the **appetite for specific foods** remained high over time and was independent of passive or active smelling. All studies using passive exposure also found appetizing effects for the cued food.[36-41] One study that used active smelling found a decrease in odour pleasantness, which was attributed to olfactory SSS. However, odour pleasantness does probably not relate to the appetite for the smelled food as found in study 3B. This is further explained in section 3 'the different definitions of SSA and SSS'.

In addition, we found that **general appetite** increased 4 mm during passive exposure to food odours (study 2A), whereas active smelling had no significant effect on general appetite or food intake of a congruent food (study 3A). Results in literature are inconsistent with respect to the influence of passive versus active smelling on appetite. After passive exposure, researchers found an increase in hunger,[36] an increase in intake[38] and a decrease in intake, depending on restrained eating score.[46] After active smelling, researchers found a decrease in appetite,[121] an increase in intake[120] and a decrease in intake, depending on weight (normal vs overweight).[44] Moreover, the size of the effects was small. There are many variables that possibly interact with appetite and intake, such as hunger,[81, 83, 84] palatability,[175, 191] similarity of test food and cue,[36] type of food cue (i.e. smell, taste, etc.)[149, 170] and individual differences in (un)restrained eating,[33, 36, 47, 150, 190, 270] BMI[44] and impulsivity.[45] The inconsistency in the results possibly reflects the small effect size and the many confounding variables. Based on the findings in studies 2A, 3A and literature, we suggest that the way of sniffing does not affect what is eaten, but possibly affects general appetite.

2.2 Switching (study 3B)

Furthermore, we investigated how **switching** between sweet and savoury odours affected general appetite and the appetite for specific foods. The appetite for specific products adjusted within one minute after a switch in odours to the new smell. This means an

increase in the appetite for the smelled food and a decrease in the appetite for incongruent foods. Unexpectedly, there were no significant effects of previous odour exposure on general appetite or the appetite for specific foods. The changes in the appetite for specific foods possibly occurred earlier than we measured, which would correspond with the faster cephalic phase responses.[e.g.194] To our knowledge, no other studies investigated the effect of switching between odours on general appetite or the appetite for specific foods. Apparently, our food preference system adapts within one minute to environmental changes.

2.3 Exposure time (studies 2A, 2B and 3A)

Another aspect studied was the impact of exposure time on general and specific appetite. We hypothesised that a one-minute exposure to food odours would increase general appetite and the appetite for specific foods,[34, 35, 37, 140] whereas a 10-20 minute exposure would decrease general and specific appetite,[44, 57, 121, 141-143, 152, 168, 169] based on literature of food cue exposure. However, the increase in general appetite and appetite for specific foods was independent of exposure time. Larsen *et al.*,[45] also found that odour exposure time, i.e. one or twenty min, did not influence food intake. The need for nutrients did not change during the odour exposure since no food was consumed. Therefore, it appears rational that the appetite for specific foods does not differ between a short or a long exposure time. However, observations from exposure therapy with obese people indicate that food cravings can diminish considerably after sixty minutes of intensive smelling, licking and tasting palatable foods.[271] Therefore, the question remains if the elevated appetite for smelled foods would drop to baseline values when the exposure time is long enough.

Interestingly, odour pleasantness decreased during odour exposure (study 3B), whereas the appetite for the smelled food remained elevated. The decrease in odour pleasantness was also found by Rolls and Rolls,[57] which they named olfactory SSS. Olfactory SSS initially sustained our hypothesis that orthonasal odours could be satiating after a longer exposure time. This apparent contraction in definition is discussed in the next section on SSS and SSA. Although there was a small decrease in odour pleasantness, the banana and meat odour were still pleasant and therefore smelling them probably still contributed to the enhancement of the appetite for those foods.

To conclude, exposure time, passive or active sniffing, and switching had no influence on the appetite for specific foods and active versus passive smelling possibly differ in their effect on general appetite.

3. The different definitions of SSA and SSS

SSA was defined in this thesis as the opposite of SSS. However, our results suggest that they possibly do not measure the same process: the elevated appetite for the smelled foods (SSA) appears to contrast with the simultaneous decrease in odour pleasantness, named olfactory SSS. The name olfactory SSS namely suggests an increase in satiation and thus a decrease in appetite (study 3B). In this section, we discuss results from this thesis and literature that so far were attributed to SSA and SSS (observations 1-8, Table 5.2), to disentangle possible different processes that underlie this apparent contradiction. In addition, data from imagining studies were included in this discussion, because imagining foods is a way of exposure to food cues without actual exposure to stimuli and without nutrient intake (obs 10-13). There is a great overlap in brain responses between perception of food during real consumption and mental imagery[185] and therefore, comparing the present results with imagining data may lead to new insights.

Possibly, the decrease in odour pleasantness and elevation in appetite for smelled food may be related to differences in liking and wanting.[2, 162, 272, 273] According to Berridge *et al*.,[2] it is possible to simultaneously increase wanting, while liking decreases. Intuitively, pleasantness ratings could relate to liking and desire-to-eat (DTE) ratings to wanting. However, the constructs of liking and wanting are not that easily separated and often it is not clear whether liking or wanting has been measured.[274, 275] Therefore, we discuss the results without spending too much attention to the possible link with liking and wanting.

Table 5.2 Overview of findings that are or could be attributed to SSA and SSS.

	Exposure
Intake	
1. **Decrease** in pleasantness of eaten foods, compared with uneaten foods. Also established for pleasantness of taste, smell, texture and sight of the eaten food.[11 12, 57-59]	All
2. **Decrease** in desire-to-eat eaten foods, compared with uneaten foods. [11 12]	All
3. **Decrease** in intake when offered monotonous diet, compared with a variety of foods.[11, 13]	All
Food cue exposure	
4. **Decrease** in pleasantness of the modified sham fed food (MSF), compared with other foods.[57, 141-143]	All
5. **Decrease** in odour pleasantness after prolonged odour exposure.[57, Study 3B]	Odour
6. **Increase** in expected pleasantness of cued food, compared with uncued food. Study 3A	cue
7. **Increase** in appetite for (DTE) cued foods, compared with uncued foods.[37, Studies 2A, 2b, 3a and 3B]	cue

8. **Increase** in the preference of cued foods, compared with uncued foods. Studies 2A and 3B	Cue
9. **Increase** in intake after smelling and/or seeing the food (2 min).[120] (not attributed to SSS or SSA)	Odour/sight
Imagining (not attributed to SSS or SSA)	
10. **No effect** on the pleasantness of the M&M's, after repeatedly imagining intake of M&M's.[185]	None
11. **Decrease** in intake of cheese, but not M&M's, after repeatedly imagining eating cheese.[185]	None
12. **Increase** in food intake after repeatedly imagining moving M&M's.[185]	None
13. **Increase** in intake after imagining its sight and smell (2 min).[120]	None

During food intake, SSS can be measured by a decrease in both pleasantness and DTE ratings of the eaten foods, compared with uneaten foods (obs 1-3; Table 5.2). The pleasantness and DTE scores are often highly correlated.[274, 276, 277] These decreases in reward are thought to affect meal termination,[197] which explains the lower intake of foods when offered a monotonous diet compared with variety. Thus, findings of SSS during food intake appear to be in line with each other.

However, findings after food cue exposure appear to contradict each other. Pleasantness of the sham fed food and odour pleasantness decreased (obs 4-5; Table 5.2), while expected pleasantness of the cued food, the DTE the cued food, the preference for the cued food and food intake increased (obs 6-9). **Perhaps, the pleasantness of the smell, taste and texture decreases upon actual stimulation of the senses** (obs 4-5). In observations 6-9 the rated stimulus was not perceived, but memorised (and probably imagined) to rate the pleasantness and DTE. In line with this suggestion, the pleasantness of the **taste** of the food was not affected, when foods were merely **smelled,** whereas the pleasantness of the **odour** decreased.[57] The pleasantness of the food was also not affected after repeatedly imagining food intake, thus without actual exposure to stimuli, even though imagining affected food intake (obs 10). Taken together, the actual stimulation of the senses may explain the decrease in odour pleasantness that was found in study 3B and by Rolls and Rolls,[57] whereas in other observations (obs 6, 10) no effect on pleasantness was found.

Additionally, **anticipation of food intake may elevate the appetite for cued foods during food cue exposure** (obs 6-9; Table 5.2), whereas food intake decreases the appetite for the eaten foods (obs 1-3). These observations indicate a difference in appetizing response between anticipation of food intake and food intake itself. Modified sham feeding (MSF) appears to be a special case of food cue exposure (obs 4). MSF is a technique where foods

are tasted and chewed, but not swallowed.[278] MSF decreased the pleasantness of the tasted food and increased satiety parameters,[152, 279, 280] just as after real food intake. MSF may activate neural circuits that are equal to those during real consumption, in contrast to anticipation.[152, 185, 279-281] In line with this reasoning, the repeated imagining of moving foods increased subsequent intake, equal to food cue exposure (anticipation; obs 12) and the repeated imagining of eating foods decreased subsequent intake, equal to real food intake (obs 11).[185] Therefore, satiation and satiety can be enhanced without the intake of nutrients. **Possibly, food cues induce anticipation of food intake**, which increases the appetite and preference for the cued food (obs 6-9, 12), whereas **the suggestion or belief that real intake had taken place** (by mental imagery, modified sham feeding or real intake) **decreases the appetite and preference for the cued food** (obs 1-4, 11).[277]

The expected pleasantness (obs 6) and the appetite for the smelled food (obs 7) were given similar ratings in study 3A. The participants possibly had difficulties distinguishing between 'How pleasant would you rate the food?' and 'How much would you like to eat the food?'.[274] Therefore, it is not clear what was measured with these questions. Intuitively, both questions measure the appetite for a food.

Since the decrease in odour pleasantness (obs 5) and the elevation in the appetite for the smelled food occur simultaneously (obs 7), we can identify which factor influences eating behaviour the most. The elevated appetite for the smelled food is in line with the findings of the FPQ. Additionally, Blundel *et al.*[21] suggested that wanting (DTE) has a much more direct effect on food intake. Perhaps wanting also has a larger effect on food preference. Therefore, we argue that the odour induced increase in appetite for the smelled foods has a larger influence on what we eat than the decrease in odour pleasantness.

In conclusion, the present results indicate that the decrease in odour pleasantness during exposure may underlie a different construct than the changes in the appetite for specific products, although until now all these observations have been explained by the opposing terms SSA and SSS.

Retronasal exposure (chapter 4)

Besides studies on the impact of orthonasal odour exposure, several studies were done to get insight in possible effects of retronasal odour exposure. During the retronasal studies, a tomato soup odour was administered via a retronasal tube in the nose, each time a sip of soup was ingested (Figure 4.1). Controlling the retronasal odour concentration and timing was needed to investigate the unimodal effect of the odour, without interference of other food properties. The experimental set-up with peristaltic pump and beeps fixed the bite size, eating rate and oral exposure time per bite and thus fixed the exposure to

the taste and mouthfeel of the soup base.[139] Therefore, we can attribute differences between conditions to differences in retronasal odour. In this section, we first discuss differences due to odour concentration and exposure time, then we address the effect of adding cream odour and finally the contribution of retronasal odour to SSS is discussed.

1. Retronasal odour exposure time and concentration (study 4A)

In study 4A, an increase in both odour exposure time and concentration decreased intake with 9% (P=0.04), but did not significantly affect subjective hunger and satiation. Ruijschop *et al.*[123] found an increase in satiation ratings of 10 mm when odour exposure time was longer (P=0.04). Together with evidence from other studies sustaining an increase in satiation after an increase in sensory exposure time,[42, 44, 103, 104] it appears likely that the extent of retronasal odour exposure indeed has a minor effect on satiation, even though the differences found by us and Ruijschop *et al.*[123] were both borderline significant (P=0.04).

However, the difference in intake due to the changes in odour release profile was circa 35 g (22kJ; study 4A), which is very small compared with the circa 9000 kJ total intake during a day.[282] As a proof of principle study, the differences in odour concentration and exposure time were maximised within the boundaries of acceptance, therefore stretching possible effects to the maximum. Therefore, the effects on satiation that can be achieved in real foods are probably even smaller. It may be argued that such small contribution of odour to satiation is not relevant for product development. However, in combination with other sensory modalities, larger effects may be possible.

Retronasal odours, taste and mouthfeel are already early after perception in the brain integrated into one flavour percept via multi-sensory integration.[14, 85] Bolhuis *et al.*[283] found a 9% decrease in intake with an increase in salt concentration. Probably an increase in either odour concentration or tastant concentration increases the flavour intensity. Furthermore, both odour exposure time and total sensory exposure time affected food intake.[52, 54, 138, 139] We suggest that the total flavour intensity and exposure time may influence the satiating capacity of foods, independent of sensory modality, i.e. taste, smell or crossmodal integration.

2. Cream odour (study 4B)

In study 4B, we investigated the effect of adding cream odour in combination with different viscosities, because they may both be associated with an increase in energy density. Adding cream odour consistently increased subjective satiation and fullness between circa 7 and 13 minutes after the start of tomato soup intake. No effect on food intake was found. The temporary effect, suggests that either adaptation caused the diminished effect of cream odour over time, or other than sensory processes overruled the perception of the cream odour after 13 minutes. Furthermore, fat perception constitutes somatosensory,[284], olfactory[285, 286] and taste mechanisms[287] and the question rises if fat perception can be simulated by triggering only one of them. However, based on an fMRI study, Frank *et al.*[131] recently suggested that olive oil extract, without fat, in yoghurt might simulate fat sensations in the brain. Another study demonstrated that daily consumption of yoghurt with olive oil extract decreased total intake and resulted in weight loss.[130] Therefore, odours may indeed affect satiation via associations with ingredients.

The present studies showed either an effect on subjective ratings or on food intake. Food intake and appetite and satiation ratings are often correlated,[27] but there are many examples where a significant effect on subjective ratings did not coincide with a significant effect on food intake, or opposite.[119, 189, 288, 289] Subjective ratings and food intake measurements appear to be two separate measures and subjective ratings cannot be translated into food intake or opposite.[146] However, large differences in intake coincide in general with differences in satiation feelings. Therefore, the inconsistency in the results as presented in this thesis and literature likely reflects the small effects. To conclude, retronasal odours might affect how much is eaten, however, the effects that were found are small.

3. Contribution of retronasal odour to development of SSS (study 4A)

The pleasantness ratings of tomato products decreased more than the pleasantness of non-tomato products after tomato soup consumption. This demonstrates the contribution of tomato odour to the development of SSS. In line with these results, Havermans *et al.*[290] found that the pleasantness of the flavour of the food decreased less when a nose clip was used during food consumption. In addition, Rolls *et al.*[13] found that intake was higher when yoghurts with different flavours were offered than when offered one flavour, but not when these flavours were similar (strawberry, raspberry, cherry). The DTE ratings, however, did not show such a crossover effect of the tomato odour. Wanting (i.e. DTE) has been suggested to have a much more direct effect on food intake than liking (i.e. pleasantness) and maybe this is also valid for food preference. Therefore, we conclude

that odours contribute to the development of SSS, but these results do not clearly support the suggestion that retronasal odours influence what we eat.

Orthonasal and retronasal

Most appetizing and satiating effects that were found in this thesis with VAS were rather small (around 5 mm on 100 mm VAS), whereas a much larger difference of 10 mm was suggested to be 'reasonable and realistic' in appetite research.[291] For the orthonasal studies, these relatively small effects were very consistent over different odours, products and studies, which underpins the reliability of the outcomes. In addition, the outcomes of the food preference questionnaire were in line with these VAS ratings. Therefore, we postulate that a 5 mm difference on 100 mm VAS is relevant. The importance of these 5 mm differences on eating behaviour in daily life remains to be determined.

Finally, the results from the ortho- and retronasal studies cannot be directly compared with each other, because they differ in calorie intake that possibly interacts with the results. The orthonasal odour exposure took place prior to food intake, whereas the retronasal odour exposure took place during food intake. De Wijk *et al.*[292] suggested that orthonasal odour perception has a larger influence on food intake than retronasal odours, because the participants in their study were influenced more by the orthonasal smell than by the flavour of the custard that they actually ingested. However, no such conclusion can be drawn from the present results in this thesis.

Methodological considerations and recommendations

The possible influences of the choices that were made for the experimental set-ups and the materials and other factors that we did not control for are discussed in the following sections.

Method of odour exposure

Participants were exposed to passively and actively smelled odours via the orthonasal route and to odours via the retronasal route administered via a retronasal tube. The passive exposure resembles situations in daily life when people experience food odours in their neighbourhood, e.g. during twenty minutes of cooking. Furthermore, the ten-minute active sniffing is less common, as in normal situations sniffing food occurs for shorter moments. The present results show that passive and active smelling have similar effects on the appetite for specific products and may evoke small differences in general appetite. Given the similarity of results, we consider the external validity of results from both passive and active exposure as good.

The retronasal experimental set-up does obviously not correspond to a real life setting, but it was necessary for studying the unimodal effect. A retronasal tube in the nose in combination with receipt of the soup via a tube in their mouth and instructions to swallow at the beep probably caused discomfort to several participants in study 4A. For reliable measurements of *ad libitum* intake, it is preferred that participants feel at ease, because they need to listen to their internal appetite feelings. The possible discomfort from the experimental set-up might overrule internal feelings of satiation and therefore affect the outcomes. To minimise the effect, we considered the first session in studies 4A and 4B as training sessions. In addition, the participants in study 4B were explicitly told they were free to stop after the first training session in order to retain only participants who experienced no or little discomfort. Therefore, we assume that the influence of the discomfort of the retronasal tube on the study outcomes in studies 4A and 4B was small.

The retronasal studies investigated the unimodal effects of odour on satiation and all other sensory processes were standardized. However, the slow eating rate possibly emphasised the exposure to sensory signals compared with normal eating and, therefore, the effects of the retronasal odour exposure on satiation were possibly overestimated. The effects of retronasal odour exposure on satiation in a natural setting remain unclear.

Using the olfactometer allowed us to study the effect of odour concentration in a controlled manner. On the other hand, the odour solutions in the olfactometer become depleted during usage. The depletion rate depends on the volatility of the odorants. We aimed to diminish odour depletion as much as possible by renewing the odour solutions every four sips, approximately after 2 minutes. Furthermore, we used a highly concentrated odour solution, in combination with a very low flow rate for the odorized air, to keep depletion as low as possible. However, as seen in Figure 4.2, there was still depletion during the experiments. This depletion occurred in all conditions in a similar way and therefore, we find it legitimate to compare the conditions with each other.

Measuring the appetizing and satiating effects of odours

Much of the drives for eating behaviour are unconscious. It is thought that such unconscious behaviour is caused by psychological attributes in an automatic manner and can better be approached via an implicit measure, for example via observation of food intake.[232] In the present thesis both explicit (VAS ratings) and implicit measures (*ad libitum* intake) were taken. In general, wanting is seen as a predictor of food intake and *ad libitum* intake is considered an implicit measure of satiation.[293] However, Griffioen-Roose *et al.*[294] observed that food intake in a natural environment correlated best with implicit measures of wanting, but in a lab setting with explicit wanting. The setting in the lab of the present

studies, especially the retronasal studies, may have changed the implicit character of *ad libitum* intake to a more explicit measure and therefore, these results should be interpreted with caution.

Furthermore, VAS ratings were used to monitor a number of appetite feelings. These explicit ratings address conscious feelings and may not represent actual behaviour.[21, 146] The change ratings from the eaten or smelled foods were compared with change ratings of the reference products to determine SSA and SSS. In the orthonasal studies, exposure to sweet and savoury odours had no or very little influence on the appetites for the reference products. During exposure to odours alone, there is no food intake and therefore, it is debatable if the appetite for the reference products is needed for a proper determination of SSA. However, the bread odour increased the appetite for all foods (not all significant, study 2A), and comparison with the appetite for reference products is in this case needed to specifically determine the effect on the smelled food. The calculated values for SSA and SSS depend on the chosen set of reference products and these should be chosen with care. In all studies, the type of reference products were matched with the type of smelled products.

Furthermore, food preference was assessed with the food preference questionnaire (FPQ), for which participants repeatedly chose between foods. The advantage of this computerised questionnaire is the high number of choices that can be measured, providing a better overview of the effects of the odours. It is not clear how well these results correspond with choices in daily life. However, the consistency in the outcomes for appetite for specific products and FPQ increases the reliability and validity of both results.

Finally, in the introduction (chapter 1), we explained that we consider SSA and SSS as measures for what is eaten. However, this probably applies to a situation where there is a variability of foods. When there is only one food available, as in studies 3A, 4A and 4B, the desire-to-eat that one product diminishes during eating and food intake may stop due to hedonic reasons. Hetherington *et al.*[197] found that people stop eating for two reasons: hedonic reason or feeling full. In this particular situation, SSA and SSS may influence how much is eaten. Therefore, it is debatable if the *ad libitum* intake that was measured in the present studies is a measure for satiation, as we suggested in chapter 1, or for sensory-specific satiation.

Experimental design

In all studies, we used a within-subject crossover design, with repeated measures over sessions. We found a larger variability in the data of the first sessions, especially in the retronasal studies. Therefore, we incorporated a training session to familiarize the

participants with the set-up and the questions. No training session was included in studies 3A and 3B, because the effects found in studies 2A and 2B during orthonasal exposure were small. Furthermore, all participants in the present studies were scheduled on the same time of the day after having a standardised breakfast, ruling out possible effects of hunger or circadian rhythm in appetite[61, 295] or olfactory function.[296, 297] Hunger increases food cue reactivity[81-84] and modulates food preference.[298-300] Possibly in a satiated state the effects of odour exposure on general appetite and the appetite for specific foods would have been smaller. This is interesting, since eating in the absence of hunger is seen as one the causes of over-eating.[301] Moreover, participants reported being a bit bored in between questions. However, we chose not to distract the participants during waiting times, because distraction has been regularly found to affect feelings of hunger and satiation and food intake.[63] The order of the test conditions was slightly unbalanced in some studies, because not all conditions could be offered on each day. However, we used statistics that can deal with unbalance in the data and missing data.

In the orthonasal studies, a control condition with no-odour was included. Without control condition, such data are easily misinterpreted by assuming the change from baseline is caused by the odour. The present data show that appetite changes over time also in the no-odour conditions, probably due to answering the appetite questionnaire. However, it may also be argued that the effects on general appetite as found in the present studies are smaller than the actual effects, because the control condition included exposure to food cues in the form of questions. The effects as shown in the present studies are effects of odour on top of effects of answering questions.

Participant selection

In all studies, we recruited normal-weight, healthy women. We aimed for recruitment of a homogenous group of participants instead of a reflection of the population to increase the chance of finding differences between conditions. Each person is unique with a unique set of characteristics and some of these characteristics may have influenced the present outcomes without our knowledge. For example, visual imagery ability affects food cue reactivity to odours.[120] Other factors that have been suggested to affect food cue reactivity are: (un)restrained eating,[33, 36, 47, 150, 190, 270] self-control,[302-304] impulsivity,[45] over/normal-weight, [37, 44] gender[41, 305-307] and chronic stress.[308] Restrained eaters were excluded from studies 2A, 2B, 4A and 4B. Results from studies 3A and 3B did not show a correlation between any of the outcome measures and score on restrained eating (data not shown), which may mean restrained eating did not affect the outcomes in the orthonasal studies. Self-control and impulsivity were not accounted for, because there is a limit in the number of questions that can reasonably be asked. Furthermore, inclusion

criteria were <45 y and non-smoking, to avoid people with olfactory deficits as much as possible.[222, 309]

Odours and products

In the orthonasal studies (Chapters 2 and 3), a variety of odours was used to provide a broad perspective on the appetizing and satiating effects of odours over time. Prior to the orthonasal studies, we selected odours based on the following criteria: perceived well, liked by the majority of people and associated with the products that they were supposed to represent. For example, fish was not liked enough and lemon was associated with cleaning instead food. In studies 3A and 3B we used the same odours as in study 2A to increase the comparability of the results. Furthermore, the reference products in the appetite questionnaire and the food preference questionnaire were matched with the smelled foods. For example, the chocolate odour matched with the brownie and bread bun with chocolate sprinkles, which were compared against apple pie and bread bun with jam. In study 3A, the same reference products were chosen as study 2A to increase the comparability of the results. In study 3B, a slightly different set of reference foods was chosen to increase the validity of the results. Summarized, the odours, smelled products and reference products were carefully chosen to optimally investigate the effect of odours on general appetite and SSA.

For the retronasal studies (chapter 4) we used tomato soup as test product. We chose a liquid to facilitate retronasal delivery at the moment of swallowing and simultaneously measure *ad libitum* intake. Furthermore, tomato soup is popular, commonly consumed at lunch, familiar to the participants and seen as a food instead of a drink. A number of participants rated the pleasantness of the soup as low. However, the results are similar with and without these participants and therefore we assume that pleasantness did not play a major role on the study outcome.

Test environment

Odours and other food cues are everywhere, which is a complicating factor in odour research. The data in the present thesis show that already a brief odour exposure changes appetite and food preference. The orthonasal studies were conducted in four well-ventilated clean rooms, which are, however, situated above a restaurant. Brief odour exposures by walking past the restaurant might have cued participants before the experiment. However, we found no indications that the time of the day affected any of the outcomes (study 2A), while the restaurant was not open during the early sessions of a test day. Therefore, we assume that the odours from the restaurant had only small effects on the outcomes. Furthermore, we took precautions to prevent odour contamination in

the test areas, by differentiating between experimenters that handled the odours and experimenters who were in contact with participants or the test areas. A sensory room with overpressure and filtered air is ideal for this type of research.

Statistical approach

All data in the orthonasal studies consist of repeated measures due to the within-subject designs. In addition, the appetite and specific appetite ratings were repeated over time during one session, which increases the complexity of the datasets. Linear mixed models are very suitable for analysis of this type of data and choosing covariance structures that fit the data well increases the reliability of the P-values. The reason of the complexity of the datasets is the different covariance structures that are needed for different levels of the data. For example in study 3B, the appetites for four sweet foods (mango, tompouce, M&M's and strawberry yoghurt) were scored at each of five time points (0, 1, 5, 7 and 11 minutes) in four sessions, resulting in 4x5x4=80 repeated measures per participant for the appetite for sweet. Similarly, the appetites for banana, meat, savoury and staple foods were repeatedly scored. In order to analyse the effect of a food odour on the appetite for specific foods, the correlation between sweet and savoury products was allowed a different coefficient (found to be negative) than the correlation between sweet and banana products (found to be positive), by using an unstructured covariance matrix for this part of the model. The data between separate time points fits best with an autoregressive covariance structure. This means that data closer in time have a higher correlation than data that have a longer time in between measurements. Then at the participant level, the covariance structure that we used was compound symmetry, which means that all variances are assumed to be equal and also all covariances have the same value. For the factor session also compound symmetry was assumed by including it as a factor with a random effect. The data in studies 3A and 3B were analysed using the approach as mentioned above. The data in studies 2A and 2B were analysed by using compound symmetry at all levels and the appetites for sweet, savoury and staple foods were analysed separately. This is a much simpler approach. The P-values generated in studies 2A and 2B, however, show highly significant differences and therefore a more complex statistical approach would probably not change the conclusions. The data from the retronasal studies do not contain such a complex data structure and analysis with one covariance structure is proficient.

Implications of results

Limiting food stimulation

The current studies clearly show that exposure to all kind of food odours change food preference and may increase appetite. Food cues in general influence eating behaviour. Dieting, for example, becomes more difficult when food cues are around and more likely unhealthy snacks with a high caloric density are chosen.[310] Although other factors such as genetics are also important in the development of obesity,[311] many people support the view that the current environment with the many exposures to food cues and the abundant availability of foods is a major cause of the increase in obesity. Genetics have barely changed in the past century, whereas the food environment has. The number of food outlets in an area was for example associated with BMI in the USA.[312] Knowledge about the contribution of food cues could be a motivation to limit food cues in the environment, in order to change our obesogenic environment toward a neutral environment, e.g. in and around schools.

Satiety enhancing foods

It can be questioned whether changing odour properties to increase satiation has the potential to restrict food intake on the long run. First of all, the effects of retronasal odours on satiation were borderline significant and small and should be interpreted with caution. There are many other factors that are more important in weight control that likely overrule the possible small effects of changes in odours. Second, people might learn the satiating capacity of the new foods, and adjust their intake.[313] Third, the increase in satiation as demonstrated in the present thesis might reflect an undesired feeling of being tired of the food instead of fulfilment.

Apart from the above mentioned restraints, it should be questioned whether an increase in the satiating capacity of foods by merely changing the odours is desirable. It has been found that using light products, by usage of sweeteners or fat replacers, disrupts food intake regulation.[314, 315] The associations between the sensory properties of foods and the metabolic consequences are very useful for weight-control and it is advisable to keep them intact. However, changing food properties in such way that exposure to flavours is extended, e.g. by using a firm texture,[219] might keep the associations intact and can be useful for weight-management.

Suggestions for further research

The present thesis focussed on the influence of odours on appetite and satiation of participants in a moderately hungry state. Eating in the absence of hunger may be one of the problems causing overeating. Therefore, future research may focus on the effect of odours in a satiated state.

Odour-nutrient relationships

We hypothesised that food odours provide information about the nutrient composition of their associated foods, with savoury odours linked to protein and sweet odours with sugar content.[187] Staple foods and staple odours appear to be a separate category that contains mainly non-sweet carbohydrates and account for a large part of our food intake.[269] Exploring the association of odours with presumed nutrient content may provide information about the role of odours as communicator of nutrient content. Van Dongen *et al.*[28] revealed taste-nutrient relationships by offering a set of 50 different foods to participants who rated their expectations on content of fat, proteins, carbohydrates, sugars and salt. A similar experiment with exposures to different food odours could reveal possible odour-nutrient relationships of common foods. This may be extended with other associations of food odours, for example by exploring how satiating the associated food is expected to be.

The influence of odour and/or food type on the appetite for the smelled food

We suggested that the congruency between an odour and a food is a graded scale. Perhaps, the congruency between the odour and a food influences the effect of the odour on the appetite of that food. However, this relationship may be more complicated, with interactions of the nutrient composition and the pleasantness of those foods. Several studies showed that the pleasantness of fatty foods and their odours were more susceptible to changes in hunger than non-fatty foods.[60, 316, 317] After seeing a picture of high caloric food, participants were quicker in doing a task that was unrelated to the pictures, compared with seeing low caloric food.[318] Whether or not fatty products are also more susceptible to odour exposure could be further explored.

Furthermore, the effect of odour pleasantness on the appetizing and satiating effects of odours could be explored. Rogers and Hill[33] found higher hunger and salivation after cues of palatable foods, than of non-preferred foods. In addition, Mattes[319] found that pleasantness of the food influenced physiological responses that affect satiety and therefore may also influence the appetizing response to a food cue. Therefore, we

hypothesise that exposure to pleasant odours increase the appetite for the smelled products and unpleasant odours decrease the appetite for the smelled product.

Investigate the effects of individual differences in food cue reactivity

It has been suggested that individuals differ in their food cue reactivity, depending on BMI,[37, 44] impulsivity,[45] self-control,[302-304] restrained eating,[27, 30, 39, 133, 180, 258] gender[35, 281-283] etc. Two contradicting theories exist about the relationship between food cue reactivity and food intake. It is proposed that hyper-responsitivity to food cues increases the risk for overeating, whereas others postulate that hypo-responsitivity results in overeating to compensate the lack of reward.[for review see 320] In order to fine-tune the appetizing and satiating effects of food cues, it is necessary to take more individual differences into account, for instance by building a model with all possible variables. Alternatively, a large group of people (e.g. 300) could be tested on food cue reactivity in combination with an extensive set of questionnaires regarding all kinds of traits or behaviours for a first screening of possible other confounders.

Conclusions

Orthonasally smelled odours affect to a larger extend what you eat, than how much you eat. They influence the appetite for specific foods via a typical pattern: the appetite for the smelled foods and for congruent sweet or savoury foods increases, whereas the appetite for incongruent sweet or savoury foods decreases. This typical pattern is **independent of exposure time, passive or active smelling and switching** between odours. The reason for this pattern is unknown, however, it may be caused by the preparation of the body for the intake of the smelled food, as food odours may provide information about the nutrient composition of their associated foods. Furthermore, **passive** odour exposure may enhance general appetite (how much), whereas **active** smelling appears to have no effect. Interestingly, the appetite for the smelled foods remained elevated during the 20-minute smelling, although the pleasantness of the smelled odour decreased a little over time. This shows an earlier assumption from literature incorrect: a decrease in pleasantness of the odour does not lead to less appetite for the smelled food. This seeming contradiction may result from different mechanisms, such as a decrease in hedonic value during prolonged sensory stimulation on the one hand and anticipation of food intake on the other hand. Furthermore, food odours were found to change preference in circa 20% of the cases. Probably, food odours shift food preference, but do not overrule strong initial preferences in circa 80% of the cases.

Moreover, **retronasally** smelled odours probably have a small influence on satiation, though the evidence is not very strong. An increase in both retronasal **odour concentration** and **odour exposure time** may enhance satiation. Adding **cream odour** may temporarily affect subjective satiation but does not affect food intake. However, the satiating effects that were found in these studies with retronasal odour exposure were borderline significant and data on food intake and subjective appetite ratings were not consistent, which probably reflects the small effect size.

Orthonasal odours influence food preference and could potentially be used to encourage healthy eating behaviour. The studies in this thesis were conducted under controlled circumstances and the results possibly deviate from behaviour in daily life. Therefore, it is unclear how strong the influence of odours is on our eating behaviour in daily situations. Finally, we advise product developers not to focus on changing retronasal odour characteristics in order to enhance satiation of products, seen the small effects that were found in this thesis.

Glossary

Orthonasal: smelled through the nose.

Retronasal: smelled through the mouth.

Food cue: consciously or unconsciously perceived stimulus that is associated with food.

Cued food: The food that is associated with a food cue.

Cephalic phase responses (CPR): physiological processes that prepare the body for food intake after exposure to food cues.

Hunger: conscious sensation reflecting a mental urge to eat. Can be traced to changes in physical sensations in parts of the body – stomach, limbs or head. In its strong form may include feelings of light headedness, weakness or emptiness in stomach.[21]

Appetite or general appetite: hedonic hunger, incorporating eating in the absence of hunger and influenced by the environment.[22] In this thesis measured as the average of hunger and desire-to-eat ratings.

Desire-to-eat (DTE): desire to eat food, thought to reflect wanting.

Satiation: process that leads to the termination of eating; therefore controls meal size. Also known as intra-meal satiety.[21]

Satiety: process that leads to inhibition of further eating, decline in hunger, increase in fullness after a meal has finished. Also known as post-ingestive satiety or inter-meal satiety.[21]

Subjective satiation: satiation measured by using subjective ratings (in this thesis by using visual analogue scales).

Sensory-specific satiety (SSS) or sensory-specific satiation: the decrease in the pleasantness or DTE of eaten foods, relative to uneaten foods.[11 12]

Sensory-specific appetite (SSA): the increase in the appetite for cued foods, relative to uncued foods.

Olfactory SSS: decrease in odour pleasantness of the smelled food, relative to other odours.[57]

Appetite for the smelled (or sweet or savoury or staple) food: appetite for specifically the smelled (sweet, savoury, staple) food

Food Reward: a composite process that contains "liking" (hedonic impact), "wanting" (incentive motivation), and learning (associations and predictions) as major components. Normally all occur together but the three psychological components have separable brain systems, which permits dissociation among them in some conditions.[2]

Flavour: the perception of a food by smell, taste and trigeminal stimuli.

Odorant: any specific aromatic chemical.

Odour: a general smell sensation of a particular quality.

Olfactometer: device used for producing odours in a precise and controlled manner.

Modified sham feeding (MSF): is a technique where foods are tasted and chewed on, but not swallowed.[278]

Visual analogue scale (VAS): line scale for subjective ratings.

Food preference questionnaire (FPQ): a computerised questionnaire that showed pairs of food pictures to participants, who had to choose the food that they would like to eat the most at that moment, based on work of Finlayson *et al.*[90, 91]

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Samenvatting

Summary in Dutch

Achtergrond en doel

Ongezonde eetgewoonten, zoals ongezonde voedselkeuzes of overeten, verhogen de prevalentie van obesitas, diabetes, kanker, cardiovasculaire en andere ziekten. Daarom is het belangrijk om te begrijpen hoe afzonderlijke factoren, zoals zintuiglijke processen, ons eetgedrag beïnvloeden. Ons reukvermogen is een van de factoren die van invloed kan zijn op de regulering van voedselinname. Voorgaand onderzoek toont aan dat voedselgeuren eetlust kunnen opwekken en versterken, maar ook kunnen verminderen. Hierbij is het nodig een onderscheid te maken tussen algemene eetlust en specifieke eetlust. De algemene eetlust weerspiegelt de algemene zin om te eten en de specifieke eetlust de zin om een bepaald product te eten: bijv. de zin in een banaan of de zin in tomatensoep.

Het hoofddoel van dit proefschrift was te onderzoeken onder welke omstandigheden geuren eetlustopwekkend of juist verzadigend zijn, om zodoende factoren die van invloed zijn op ons eetgedrag te kunnen identificeren. Geuren worden via twee routes waargenomen die we beide onderzochten: orthonasaal via de neus nemen we de buitenwereld waar en retronasaal via de mond ruiken we het eten in onze mond. Doordat retronasale geur tegelijkertijd wordt waargenomen met het voedsel in onze mond lijkt het alsof we deze geuren proeven. We varieerden de blootstellingsduur aan een geur, de geurconcentratie en het geurtype. De orthonasale geuren werden verspreid in een kamer (passieve manier van ruiken) of in een bekertje gedaan waaraan actief moest worden geroken. Verder onderzochten we wat de invloed van het wisselen tussen geuren is op onze eetlust.

De onderzoeken

In totaal deden we zes experimenten waarbij we vrouwen met een normaal gewicht lieten ruiken aan verschillende geuren.

In de eerste twee experimenten (hoofdstukken 2A en 2B) werden geuren in een kamer verspreid. Dit noemen we een **orthonasale passieve** manier van ruiken. In deze geurkamer vulden deelnemers vragen in over hun eetlust en over de specifieke zin in een twintigtal verschillende voedselproducten. Aan het eind kregen de deelnemers foto's met eten te zien waarbij ze telkens het eten moesten kiezen dat ze het liefst zouden eten. Dit is óók een maat voor specifieke eetlust. De resultaten laten zien dat blootstelling aan voedselgeuren de algemene eetlust een beetje verhoogt. Geuren verhoogden specifiek de zin in het voedsel dat geroken was, maar verminderden de zin in producten met een heel andere geur: zoete geuren verhoogden de zin in zoete producten, maar verlaagden de zin in hartige producten en vice versa voor hartige geuren. De invloed van de geur op de algemene en specifieke eetlust veranderde niet gedurende de 20 minuten durende

blootstelling, terwijl we juist verwacht hadden dat de zin in het geroken product zou dalen met de tijd. Niet-voedselgeuren leken de eetlust iets te onderdrukken. De voedselkeuze veranderde in ongeveer 20% van de gevallen bij het ruiken van een voedselgeur.

In de twee volgende experimenten met **orthonasale** geur (hoofstukken 3A en 3B), werd de geur niet passief in de kamer verspreid, maar moesten deelnemers **actief** ruiken aan een bekertje dat gevuld was met water (controle), bananengeur of vleesgeur. Dit bekertje was afgedekt met een zakdoekje zodat ze niet konden zien wat er in zat. Eetlust wordt namelijk ook beïnvloedt door wat we zien. De deelnemers moesten weer vragen invullen over hun algemene en specifieke eetlust voor en tijdens het ruiken. In hoofdstuk 3A kregen de deelnemers na het ruiken nog enkele broodjes met een grote hoeveelheid bananenmilkshake, waarbij gemeten werd hoeveel ze aten. Verder werden in hoofdstuk 3B de bekers met geur na 5 minuten gewisseld voor een beker met een ander geur om te onderzoeken of de eerder geroken geur nog invloed heeft wanneer een nieuwe geur wordt geroken. Wederom zagen we dat de zin in het geroken product toenam en de zin in voedsel met een andere geur afnam. Dit patroon paste zich binnen een minuut na geurwisseling aan de nieuwe geur aan (hoofdstuk 3B). Actief geroken voedselgeur had, in tegenstelling tot passief geroken geur, geen significant effect op de algemene eetlust of op de hoeveelheid die gegeten werd (hoofdstuk 3A). Verder zagen we een kleine daling van de aangenaamheid van de geur tijdens het actieve ruiken, terwijl de zin in het geroken voedsel hoog bleef (hoofdstuk 3B).

In de laatste twee experimenten werd een **retronasale** tomatensoepgeur door een slangetje in de neus geblazen tijdens het eten (hoofdstukken 4A en 4B). Dit slangetje werd 8 cm in de neus gebracht om zo retronasale geurwaarneming te realiseren zonder dat deze waarneming werd beïnvloed door smaak en textuurwaarneming in de mond. Met een olfactometer kon de geurconcentratie precies worden gereguleerd. Via een andere slang werd een soepbasis in de mond gepompt net op het moment dat er geur in de neus werd geblazen. De soepbasis en de geur samen gaven de perceptie van tomatensoep; het leek alsof de geur uit de soepbasis kwam. Naast vragen over eetlust werd ook gemeten hoeveel van de ‘tomatensoep’ gegeten werd. In hoofdstuk 4A werden de blootstellingsduur aan de geur en de geurconcentratie gevarieerd. De resultaten wezen uit dat meer geur (zowel langere duur als hogere concentratie) leidt tot iets minder eten, maar dit had geen significant effect op hoe vol de deelnemers zich voelden. Ook werd de bijdrage van geur aan de vorming van sensorisch-specifieke verzadiging nog eens aangetoond. Sensorisch specifieke verzadiging is de daling van de aangenaamheid van een gegeten product, relatief ten opzichte van niet-gegeten producten. In hoofdstuk 4B werd onderzocht of toevoeging van roomgeur aan tomatensoep, in combinatie met een lage of

hoge viscositeit, verzadiging beïnvloed. Het lijkt erop dat het waarnemen van de roomgeur het gevoel van verzadiging tijdelijk verhoogde. Dit effect verdween echter na 13 minuten en de roomgeur had ook geen invloed op de hoeveelheid soep die werd gegeten.

Conclusies

Orthonasale geuren hebben een grotere invloed op wat je eet dan hoeveel je eet. Ze beïnvloeden de eetlust via een typisch patroon: de zin in het geroken voedsel wordt groter, evenals de zin in vergelijkbaar voedsel, terwijl de zin in voedsel met een heel andere geur daalt. Dit typische patroon is onafhankelijk van de manier van ruiken (passief of actief), blootstellingsduur of het wisselen tussen geuren. De reden achter dit patroon is niet bekend, maar het zou veroorzaakt kunnen worden door de voorbereiding van het lichaam op inname van het geroken voedsel doordat geuren informatie verschaffen over de nutriëntensamenstelling. Verder lijkt een passieve geurblootstelling de algemene zin om te eten iets te verhogen, terwijl actief ruiken geen effect heeft. De zin in het geroken voedsel blijft hoog gedurende ten minste 20 minuten blootstelling aan geur, ondanks een kleine daling in de tijd van de aangenaamheid van de geur. Hierdoor blijkt een eerdere veronderstelling uit de literatuur onjuist; een daling van de aangenaamheid van de geur leidt niet tot een verminderde eetlust in het geroken product. Deze schijnbare tegenstelling zou kunnen worden verklaard door een afname van de hedonische waarde bij een langdurige sensorische blootstelling, terwijl de geuren tegelijkertijd de eetlust verhogen door anticipatie van voedselinname. Voedselgeuren bleken de voedselkeuze in 20% van de gevallen te veranderen. Dat de keuze in 80% van de gevallen onveranderd bleef zou kunnen worden verklaard door sterke persoonlijke voorkeuren.

Retronasale geur heeft waarschijnlijk een kleine invloed op het optreden van verzadiging, maar de gevonden effecten zijn niet overduidelijk. Verhoging van retronasale geurconcentratie en verlenging van de blootstellingsduur zou verzadiging kunnen verhogen. Ook het toevoegen van roomgeur zou het verzadingsgevoel tijdelijk kunnen verhogen. De gevonden significante effecten waren echter maar net significant en bovendien waren de resultaten tussen verschillende metingen niet consistent.

Orthonasale geuren zouden mogelijk gebruikt kunnen worden om gezonde keuzes te bevorderen. De studies in dit proefschrift zijn uitgevoerd onder gecontroleerde omstandigheden en de resultaten wijken mogelijk af van gedrag in het dagelijks leven, waardoor het nog onduidelijk is hoe sterk de invloed is op ons eetgedrag. Gezien de kleine effecten van retronasale geur op verzadiging, adviseren wij productontwikkelaars om zich niet te richten op het aanpassen van retronasale geureigenschappen om verzadiging te vergroten.

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About the author

Mariëlle van den Dungen – Ramaekers was born on June 12th, 1976 in Asten, the Netherlands. After her secondary (VWO) at College Asten Someren she started her study 'Food Technology' at Wageningen University, with a major in Food Physics and an internship about the behaviour of micro gel particles in different polymer solutions at Unilever Research Colworth House in England. She started her first job at International Flavors and Fragrances (IFF) in Hilversum, the Netherlands, where she discovered her interest in sensory science and human behaviour by working with sensory panels in the Sensory and Consumer Insights department. Then she worked for the customer service for Silliker BV in Ede in the Netherlands, after which she decided to take an extra year of studies with courses on topics about statistics, communication, consumer behaviour, sociology, cognitive neuroscience and other courses at Wageningen and Utrecht University. In 2007 she started her PhD in a topic related to sensory science.

List of publications

Ramaekers MG, Boesveldt S, Lakemond CMM, van Boekel MAJS, Luning PA. Odors: appetizing or satiating? Development of appetite during odour exposure over time. *International Journal of Obesity* 2014; 8(5): 650-656.

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Overview of completed training activities

Discipline specific courses

Course 'Food perception & food preference', 2007, Graduate school VLAG, Wageningen, The Netherlands

Course 'Perception and Action', 2007/2008, Utrecht University, Utrecht, The Netherlands

Course 'Smell and taste', 2008, University of Dresden, Germany

Summer School Human olfaction, 2011, University of Dresden, Germany

Conferences and Meetings

Weurman symposium (12th), 2008, Interlaken, Switzerland (poster presentation)

ECRO conference, 2008, Portoroz, Slovenia (oral presentation)

Pangborn symposium, 2009, Florence, Italy (poster presentation), received **student bursary award**

Annual meeting of the British Feeding and Drinking Group, 2010, Maastricht, the Netherlands (oral presentation)

Annual meeting of the British Feeding and Drinking Group, 2011, Belfast, the Netherlands (poster presentation)

STW symposium linking product properties to obesity prevention, 2012, Wageningen, The Netherlands (oral presentation)

Minisymposia, 2008-2010, Wageningen Library

Discussion group meetings 'Eetclub', 2007-2012, human nutrition, The Netherlands

General courses

PhD competence assessment, 2007, Wageningen Graduate Schools, The Netherlands

'PHD introduction course', 2008, Graduate school VLAG, The Netherlands

Courses 'Introduction and advanced Literacy', 2008, Wageningen Library, The Netherlands

Course 'Scientific Writing', 2008, Wageningen Graduate Schools, The Netherlands

Course 'Linear mixed models (statistics), 2009, Graduate school WIAS, The Netherlands

Workshop 'How to write a world class paper', 2010, Wageningen Graduate Schools, The Netherlands

Course 'Career perspective', 2012, Wageningen Graduate Schools, The Netherlands

Optionals

Participating in the PhD study trip to Australia, 2012

Preparation of research proposals, 2008-2013

Research presentations, 2007-2012

Colophon

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