SALIVA AND SENSORY PERCEPTION

INTERPLAY BETWEEN THE PERSON AND THE FOOD STIMULI

CATHRINE I. HEINZERLING

Thesis Committee

Promotor

Prof. Dr G. Smit
Professor of Molecular Flavour Science

Wageningen University

Co-promotor

Dr J.H.F. Bult
Senior Scientist Perception & Psychology, NIZO Food Research BV, Ede

Other members

Prof. Dr C. de Graaf, Wageningen University
Prof. Dr A Vissink, University of Groningen
Dr J. Ley, Symrise, Holzminden, Germany
Dr R.W Visschers, TNO, Utrecht

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Saliva and sensory perception
Interplay between the person and the food stimuli

Cathrine I. Heinzerling

Thesis

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How do you eat an elephant?

Piece by piece

Tack Oliver!

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Saliva and Sensory

Interactions between the Person and the Food Product

Key words: sensory, saliva, interactions, taste, texture, perception

Abstract

The perception of food is influenced by various parameters, many of them being different from individual to individual. What we perceive is not the same because each individual is different. Saliva volume and composition vary widely among people and will influence the chemical and structural composition of the food. Thus, the dilution and mixing of the food with saliva determines the extent of food-saliva interactions and connected to that also how the food item is perceived. It is clear from literature that saliva affects our perception and it is also clear that the rate and composition of salivation is dependent on what we perceive. However, it has not been clear to what extent. Since saliva can be measured objectively for each individual and it can be manipulated in a controlled fashion, more can be learned from the relationship between oral processing and perception. And with that various questions can be addressed, such as: Can the individual differences in sensory assessment be accounted for by their individual salivary composition? Is it possible to affect the sensory perception of an individual by modifying their salivary flow and composition? Different tastes stimulate different amounts of saliva but do they also affect the saliva composition? Or are the differences in saliva composition caused by the differences in salivary flow rate? Can different amounts of saliva, and thus also different dilution factors, affect the taste perception? Furthermore, can taste-taste interactions be explained by an increase in salivary flow rate? Is it possible that the increased salivation, induced by the increased thickness, will dilute the tastant and hence decrease the perceived intensity? Or are taste-texture interactions caused by cross-modal interactions? Or is the increased viscosity of the texture decreasing the concentration of taste molecules? The aim of this thesis is to show how and to what extent saliva influences, and is influenced by, taste and texture.

The addition of amylase inhibitor reduces saliva α-amylase activity and increases perceived thickness and creaminess. However, alpha-amylase activity varies widely among subjects and therefore a decreased oral α-amylase activity will not guarantee an increase in perceived thickness and creaminess of starch-based foods. Comparisons of the different tastants show that the pH of stimulated parotid saliva increases linearly, irrespective of the nature of the tastant. Protein concentration decrease and protein amount increase with increase in flow rate for all tastants. After correcting for the effect of flow rate, the protein amount is affected by the nature of the tastant with the greatest secretion after stimulation by citric acid. Flow rate is largely responsible for pH but tastant appears to play an additional role in affecting protein secretion. Significant decreases in perception with increasing salivary flow rates are observed for citric acid and sodium chloride. This can partially be explained by a dilution effect which is in line with previous studies on detectable concentration differences. However, since the bitterness and sweetness remain unaffected by the salivary flow conditions and the dilution effect is comparable to that of saltiness, further explanations are still needed.

Suppression of taste intensity in binary mixtures is not affected by the rate of salivation. This is more likely explained by psychophysics. When the taste is separated from the texture, no texture-taste effects are observed. Dilution with saliva did occur and the tastant availability was unaffected in this set-up. The conclusion is therefore that texture-taste interactions are not caused by dilution effects or cross-modal interactions but can best be explained by the release of tastants.

The work described in this thesis shows how the individual perception can be affected by the salivary flow and composition and how the individual salivary flow and composition can be affected by the sensory stimuli taste and texture.

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Chapter 1

General introduction

The recommended daily food intake lays on average between 2000 and 2500 kcal per person per day, for women and men, respectively. However, we consume between 2803 and 2940 kcal per capita per day in the world (WHO/FAO 2003). We obviously eat not only because we need to but because we like to. The way the food is perceived is very important, perhaps the most important criteria for consumption, at least in the developed countries. If we do not like it, we do not eat it.

Overweight and obesity are becoming serious problems in the developing -and nowadays also more and more in the underdeveloped- countries, resulting in an increased number of persons with diabetes and ischemic heart disease. One-and-a-half billion adults and nearly 43 million children are overweight. Of these, over 200 million men and nearly 300 million women are obese (WHO 2012).

The pressure on the food industry is high to reduce sugars, salts and fat in food in an attempt to tackle the weight gain problem. However, reducing sugars, salts and fats in food and still maintain an equally-liked taste and texture is a challenge. When sugars, salts and fats are reduced it influences the complete perception and structure of the food product, including flavour (both taste and aroma) and texture. To some extent this can be compensated for by substituting for example sugars with sweeteners and sodium chloride with potassium chloride. However, these other substances often come with a bitter after taste. Another method is to enhance the taste and texture perception by using congruent aromas (Bult et al. 2007; Lawrence et al. 2009; Schifferstein and Verlegh 1996; Stevenson et al. 1999). This is however a highly complex method as congruent aromas has to be found for each specific food product. It is also culture and individual dependant as it is a conditioned effect. Furthermore, it does not compensate for the change in texture. Other studies have showed that a pulsed delivery can enhance the perception of sweetness (Burseg et al. 2010; Busch et al. 2009; Meiselman and Halpern 1973). But also this is subject to inter-individual differences. How aromas and tastes are delivered is affected by the release of taste and aroma from the product. The release can be affected by the food texture but also by the oral conditions (Ferry et al. 2006a; Ferry et al. 2006b; Hollowood et al. 2002; Koliandris et al. 2008). How the food product is manipulated in the mouth, the dilution with saliva and the taste and olfactory morphology – all affects the release. And

again these are subject to inter-individual differences. A better understanding of the mechanism and inter-individual differences behind food perception is therefore of great importance for a successful reduction in sugars, salts and fats.

1.1 Saliva

That saliva plays an important role in food perception is illustrated by the proverb 'makes your mouth water', which is present in almost all languages. Everyone knows the meaning of this quote. You are hungry and just the bare thought of your favourite food makes you start drewling. Saliva will interact with the chemical properties in the food as soon as the food is put in our mouth; saliva will break down the food structure and dilute the tastants (de Wijk et al. 2004; Ferry et al. 2004; Guinard et al. 1997; Matsuo 2000; Van Nieuw Amerongen et al. 2004; Weel et al. 2002). These changes will affect the chemical and physical properties of the food material and how we perceive the sensory properties (Bonnans and Noble 1995; Christensen et al. 1987; Ferry et al. 2004). Because the salivary flow and composition is individual dependant it is important to remember that in fact each individual will taste a different food product. Even though the food product might be the same outside the mouth it will change according to the individual once it enters the mouth. Saliva is thus a major unknown contributor in taste and texture perception.

1.1.1 Function, flow and composition

Saliva has a multifunctional role. On one hand it assures the antimicrobial climate necessary for teeth maintenance, on the other hand it is also vital to digestion and food perception (Mese and Matsuo 2007). The saliva is produced mainly by three pairs of major salivary glands (Figure 1.1); the parotid (PAR), submandibular (SM) and sublingual glands (SL) but also by minor glands present on the inside of the lips and the cheeks, on the palate, and on the tongue, such as the von Ebener's glands (Matsuo 2000; Mese and Matsuo 2007; Van Nieuw Amerongen et al. 2004).

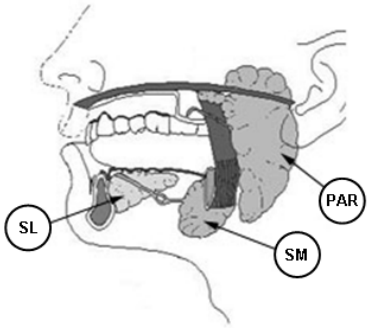

Figure 1.1 – The three main pairs of salivary glands and their ducts (Image: http://en.wikipedia.org/wiki/Salivary_gland)

The submandibular glands and the sublingual glands are located under the tongue and produce viscous, mucin-rich saliva. The parotid gland is located behind the ear, with the duct on the inside of the cheek. Its saliva is very thin and watery and rich in α-amylase (Van Nieuw Amerongen et al. 2004).

The relative amount of saliva secreted from the different glands is dependent on a number of factors. Parotid saliva, for example, does hardly contribute at all to the unstimulated saliva volume. However, upon mechanical or gustatory stimulation it stands for more than half of the total secreted saliva volume. The sublingual gland is mainly producing saliva during rest conditions and the minor glands contribute with less than 10 % to the total saliva volume in the mouth (Table 1.1) (Van Nieuw Amerongen et al. 2004).

Table 1.1 – Contribution of the various glands during different conditions (in percentage) (Van Nieuw Amerongen et al. 2004)

Glands	Sleeping	Unstimulated	Mechanical stimulation	Gustatory stimulation [2% citric acid]
PAR	0	21	58	45
SM	72	70	33	45
SL	14	2	2	2
Minor	14	7	7	8

Judging from the saliva amounts, the submandibular, sublingual and minor glands are mainly involved in oral health maintenance, whereas the parotid gland is predominantly involved in the digestion.

Saliva is secreted according to a circadian rhythm with different flow rates on different times of the day. It is also influenced by individual differences such as; salivary gland size, physiological status, age and gender. The individual differences in flow rate for parotid saliva range between 0.1 mL/min and 7 mL/min Humphrey and Williamson 2001.

Parotid saliva is one of the most investigated saliva types since it can be collected with the help of a Lashley-cup or Carlson-Crittenden-cup (Figure 1.2). The Lashley cup is positioned over the Stensen duct (parotid duct), which is located on the mucosa on the inside of the cheek, at the level of the second upper molar (Lahley 1916). The Lashley cup is kept constant with the help of vacuum and is thus a non-invasive, pain free method to exclusively collect parotid saliva.

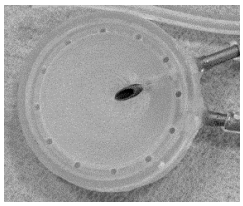

Figure 1.2 – The Lashley-cup is about 2 cm in diameter and fixated with vacuum (air sucked through the small holes on the edge). The saliva exits through the tube located in the middle of the cup

The parotid saliva consists of a variety of electrolytes and proteins (Table 1.2). The difference in concentration between unstimulated and stimulated parotid saliva is caused by the increased salivary flow (Van Nieuw Amerongen et al. 2004). The pH of saliva lies normally between 6.5 and 7.0 and its large amounts of bicarbonate ions has a buffering action on acids (Christensen et al. 1987; Larsen et al. 1999; Wakim et al. 1969). Amylase is secreted mainly from the parotid gland, making up almost 30 % of its total protein concentration (Van Nieuw Amerongen et al. 2004). The other main compounds in

the parotid saliva protein concentration are proline rich proteins and proline rich glycoproteins (together about 60 %) (Van Nieuw Amerongen et al. 2004).

Table 1.2 – Composition of unstimulated and stimulated parotid saliva (Van Nieuw Amerongen et al. 2004)

	Unstimulated	Gustatory stimulus [2 % citric acid]
[mM]		
Na^+	3	60
K^+	46	20
Cl^-	31	36
Ca^{2+}	1.5	1.0
Mg^{2+}	-	0.04
HCO_3^-	1.0	30
Phosphate	15	6
CNS^-	5-6	3
[mg/100ml]		
Proteins	100-200	100-250
Lipids	-	3
Urea	-	2.5
pH	6	6.8-7.6

1.1.2 Role in taste and texture perception

Saliva is stimulated upon gustatory and mechanical stimulation, where the flow rate directly depends on the concentration respectively the hardness of the food item (Anderson and Hector 1987). Furthermore, the composition of saliva largely depends on the salivary flow rate but also, to some extent, on the type of stimulus. The bicarbonate concentration for example increases with the flow rate whereas some proteins, involved in inflammatory responses, are over expressed after stimulation by sour, bitter and umami tastants, independent on flow rate (Neyraud et al. 2006).

Saliva is necessary for the transport of taste molecules to the taste receptor and important for the mechanical and enzymatic breakdown of a texture along with the bolus formation (Ferry et al. 2004; Guinard et al. 1997; Matsuo 2000). The rate of breakdown of the texture will also have an effect of the tastant release and thus influence the perceived taste. The neutral pH and buffering action of saliva will be of importance for the perception of acid tastes and the salt levels in saliva will determine the taste threshold for salty taste (Christensen et al. 1987; Delwiche and O'Mahony 1996; Matsuo 2000; Norris et al. 1984; Spielman 1990). The individual salivary flow rate will also have an effect on the taste threshold, likely due to dilution of the taste molecules (Lugaz et al. 2005; Norris et al. 1984). Thus, the mixing with saliva changes the physical and chemical properties of the food item.

1.2 Sensory perception

Taste receptors have to be compatible with a large number of taste molecules. The taste bud, which contains the receptor for taste perception, is located in the papillae structure. Only the filiform papillae does not have taste buds (Figure 1.3) (Behrens and Meyerhof 2006).

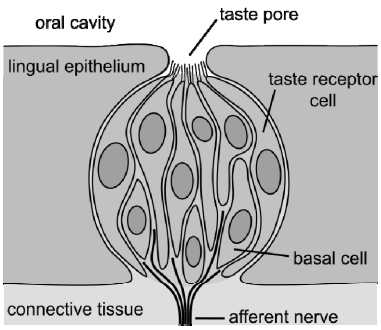

Figure 1.3 – Schematic overview of a taste bud and its different components (Image: http://en.wikipedia.org/wiki/Taste_bud)

Sugars, bitter and umami substances bind to receptor proteins and thereby activate pathways to the brain. Ionic tastants, salts and acids, activates the receptor through specific ion channels (Behrens and Meyerhof 2006).

After the taste impulse has triggered the receptor it is lead through the cranial nerves to the nucleus tractus solitarius (NTS) in the brain stem. Here the primary taste areas are located. The impulse is then lead through secondary neurons to the thalamus, which from there directs the impulse to the cortex. How the taste is coded and read is still not fully elucidated (Laing and Jinks 1996; Reed et al. 2006). Taste intensity shows a sigmoid increase with increasing concentration (Keast and Breslin 2003). The perceived intensity is assumed to have a minimum and a maximum intensity value. Below or above this value a person cannot detect any differences in taste intensity. There are two types of low taste thresholds; a detection threshold, defined by the lowest concentration of a taste stimulus that is distinguishable from water, and a recognition threshold, defined as the concentration at which the stimulus is clearly identified (Spielman 1990). To study high taste thresholds has an ethical limitation since ingesting high concentrations of tastants are highly unpleasant or even painful.

The tongue is also of importance for texture perception. Mechanoreceptors responding to tactile stimuli, similar to those in the skin, have been identified on the tongue and are thought to be present in the filiform papillae (Engelen and van der Bilt 2008). During manipulation of the food the tongue moves the bolus around in the mouth. Texture perception is thus a tactile sensation.

1.2.1 Taste-taste interactions and taste-texture interactions

Most foods are subject to taste-taste interactions and/or taste-texture interactions, meaning that the perceived intensity of one sensory attribute is influenced by the presence of another sensory attribute or by a change in the food structure (e.g., texture) (Arabie and Moskowitz 1971; Christensen 1977; Keast and Breslin 2003; Koliandris et al. 2008; Mackey and Valassi 1956; Malone et al. 2003; Pangborn 1960). For example, the perceived sweetness of sucrose is generally suppressed by the sour taste stimuli citric acid and the perceived taste intensity is generally suppressed by an increased viscosity of the food matrix (Keast

and Breslin 2003). Several explanations to these interactions have been proposed, such as cross-modal interactions, dilution by saliva or a decreased availability of tastant molecules in more viscous solutions (Bayarri et al. 2001; Boland et al. 2004; Brossard et al. 2006; Koliandris et al. 2008; Malone et al. 2003; Sala et al. 2010; Tournier et al. 2009). However, it is not clear which one of those are the most relevant.

1.3 Aim of thesis

The perception of food is influenced by various parameters, subject to large inter-individual differences. What we perceive is not the same because each individual is different. Each individual has a different taste-bud physiology, different cultural heritage and different memories (Amerine et al. 1965). The parameter saliva composes an additional source of variation, since it will change the chemical and structural composition of the food mixture during oral manipulation. Saliva volume and composition vary widely among people and it also varies during eating (Lugaz et al. 2005). Thus, the dilution and mixing of the food with saliva determines the extent of food-saliva interactions and thus also how the food item is perceived.

To what extent each of these parameters explain the intra-individual variation observed is not clear. The aim of this thesis is to look in closer detail into one of them – the role of saliva. Saliva can be measured objectively for each individual and it can be manipulated in a controlled fashion. It is clear from literature that saliva effects our perception and it is also clear that the rate and composition of salivation is dependent on what we perceive. There is a chain of interactions. The aim of this thesis is to show how and to what extent these interactions are present.

In order to do this, either the individual response is measured or the individual flow rate and composition is manipulated. The method for how this is done is also developed. The thesis is divided into four parts, each resembling one of the interactions between saliva and taste and saliva and texture (Figure 1.4).

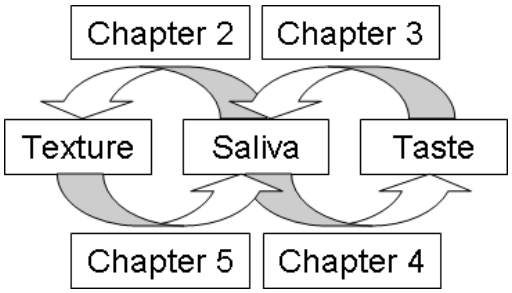

Figure 1.4 – Structure of the thesis; showing the various interactions and their corresponding chapters

1.3.1 Chapter 2 - Effects of saliva on texture

The aim is to determine the importance of salivary flow and composition on perceived thickness. Can the individual differences in sensory assessment be accounted for by their individual salivary composition? Is it possible to affect the sensory perception of an individual by modifying their salivary flow and composition? Starch-containing food can be broken down by the α-amylase in the saliva. This could possibly affect both the texture and the taste perception. If we can modify the individual salivary flow rate and α-amylase concentration, we can see how the perceived taste intensity and thickness is affected. If we use a big enough group of subjects we should also be able to see how much these individual differences in physiology account for differences in sensory perception.

1.3.2 Chapter 3 – Effects of taste on saliva

The aim is to investigate the effects of different tastants on parotid salivary flow and composition. The saliva flow and composition affects the perception but different tastes also affect the salivation. Different tastes stimulate different amounts of saliva but do they also affect the saliva composition? Or are the differences in saliva composition caused by the differences in salivary flow rate? In order to test this we need to be able to collect and measure saliva flow and composition continuously. We need to be able to relate the salivary composition directly to the salivary flow rate. In this way we can see if compositional differences are caused by the type of tastant or by the salivary flow rate.

1.3.3 Chapter 4 – Effects of saliva on taste

The aim is to determine the role of saliva flow on the taste perception. The tastant needs to be diluted and dissolved by saliva in order to be sensed and low amounts of saliva, due to old age or illness, often result in a reduced taste sensation. On the other hand individuals with a high salivary flow rate are reported to have a high taste threshold. Can different amounts of saliva, and thus also different dilution factors, affect the taste perception? Furthermore, can taste-taste interactions be explained by an increase in salivary flow rate? In order to answer these questions we need to be able to control the amounts of saliva entering the mouth. If we can then determine the amount of saliva that should be secreted, we can see how these different amounts affect the perceived intensity.

1.3.4 Chapter 5 – Effects of texture on saliva

The aim is to study texture effects on salivation and the role of saliva on taste-texture interactions. Taste perception decreases with increasing thickness of the food item. On the same time chewing stimulates salivation. Is it possible that the increased salivation, induced by the increased thickness, will dilute the tastant and hence decrease the perceived intensity? Or are taste-texture interactions caused by cross-modal interactions? Or is the increased viscosity of the texture decreasing the from taste molecules? We need to create a product with both taste and texture attributes but where the taste is not incorporated in the texture and where the taste and the texture do not chemically interact. We also need to measure the salivation rate to control for tastant dilution with saliva. If taste-texture effects do occur they can be linked to either to the dilution with saliva or to cross-modal interactions. If taste-texture interactions do not occur they are likely to be caused by a reduced tastant availability.

In the conclusion of the thesis all these aspects will be taken together to show how the work described in the chapters 2-5 contribute to our insight in the role of saliva in oral processing and finally perception.

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Chapter 2

Modelling oral conditions and thickness perception of a starch product

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C. I. Heinzerling, G. Smit, E. Dransfield (2008). Modelling oral conditions and thickness perception of a starch product, International Dairy Journal, 18, 867-873.

Abstract

Food components stimulate salivation, and the flow and composition of the saliva also affect the perception of the food product. In starch-containing foods, salivary α-amylase breaks down the starch and this may cause thinning in semi-solid foods. The aims were to determine the importance of salivary composition to perceived thickness. Vanilla custard was assessed for taste intensity, creaminess and thickness. To extend the range of saliva composition and flow, an α-amylase inhibitor was added to the samples at different concentrations and the pH of the samples was lowered by adding citric acid. From each collected spat-out bolus, temperature, pH, dilution factor and α-amylase activity were measured. Addition of amylase inhibitor reduced saliva α-amylase activity and increased perceived thickness and creaminess. Acidification increased mechanical thickness prior to testing and perceived thickness but did not reduce the in situ α-amylase activity because the saliva stimulated by acidified custards was also more concentrated in α-amylase. Alpha-amylase activity varied widely among subjects and so decreasing oral α-amylase activity would not guarantee an increase in perceived thickness and creaminess of starch-based foods.

2.1 Introduction

Starch, which contributes to the textural properties of many foods, is currently receiving interest as the nature of the starch will determine its rate of metabolism in the body and, as a result, its level in the blood (glycaemic index) and its satiating effect. Thicker foods are thought to be more satiating (Mattes & Rothacker, 2001). The link between physical and rheological properties of starches and the microstructure of emulsions is well known (Autio, Kuuva, Roininen, & Lähteenmäki, 2003); however, the link to sensory attributes is less clear. This weakness is largely due to the complexity of the physical and chemical changes occurring during eating and to the difficulty in reproducing these processes in vitro. Once the food product is present and sensed in the mouth, saliva production increases and this changes the bolus formation. Mixing with saliva changes the physical and chemical properties of the bolus, which can also influence the release and/or perception of flavours (de Wijk, Prinz, Engelen, & Weenen, 2004; Ferry, Hort, Mitchell, Lagarrigue, & Pamies, 2004; Guinard, ZoumasMorse, Walchak, & Simpson, 1997; Weel et al., 2002). Saliva has normally a pH between 6.5 and 7.0 and acts as a buffering system (Christensen, Brand, & Malamud, 1987; Larsen, Jensen, Madsen, & Pearce, 1999; Wakim, Robinson, & Thoma, 1969). The saliva contains α-amylase that hydrolyses the starch (Evans, Haisman, Elson, Pasternak, & McConnaughey, 1986; Merritt & Karn, 1977; Wakim et al., 1969) and this breakdown has been said to affect the perceived thickness (de Wijk et al., 2004). In these respects, the product acidity will be important as it will stimulate salivary flow (Engelen, de Wijk, Prinz, van der Bilt, & Bosman, 2003; Froehlich, Pangborn, & Whitaker, 1987), reduce α-amylase activity (Evans et al., 1986; Merritt & Karn, 1977; Wakim et al., 1969), and influence the perceived flavour (Guinard et al., 1997). A major unknown in this premise is the importance of the variation in the composition and flow of saliva among individuals (Christensen et al., 1987; Larsen et al., 1999).

This study aims to quantify these factors. The range of saliva composition and flow was extended by addition of an α-amylase inhibitor to the starch-based custards, at different concentrations and the pH was lowered in some of the custards by adding citric acid. From the collected spat-out bolus after assessment, the temperature, pH, dilution

factor and α-amylase activity were measured and related to sensory perception of the custards.

2.2 Materials and methods

2.2.1 Normal and acidified samples

Vanilla custard is a semi-solid, starch-based dairy product used in sensory studies. It is also common in The Netherlands to eat custard on its own as a desert. A low-fat (0.1 %) UHT-treated commercial custard (Creamex, Rijkervoort, The Netherlands) was used. For the acidified custard, 6.5 g of citric acid were mixed into 1 L of custard.

2.2.2 Acarbose

To both normal and acidified custards different amounts of an α-amylase inhibitor, Acarbose (Glucobay, Bayer, Mijdrecht, The Netherlands), were added. Three tablets, each containing 50 mg Acarbose, were crushed and shaken with 3 mL of water and then centrifuged for 3 min at 1500 *x* g using a Microlitre Centrifuge (Z 200 M/H, 230 V/50–60 Hz from Hermle Labor Technik, Wehingen, Germany). The clear supernatant was taken out and the pellet washed using the same procedure. This procedure was repeated a further 3 times. The supernatants were pooled and the volume made up to 30 mL.

From the supernatant 7 mL was added to 350 mL of the acidified custard and 7 mL was added to 350 mL of the normal custard. A further 15 mL of supernatant was then diluted to 30 mL with water. This dilution process was repeated until all five concentrations of Acarbose were present. The nominal concentration of Acarbose, assuming 100 % extraction, is used to distinguish the samples although its exact concentration is not important since the α-amylase activity was measured directly. The range of the nominal concentrations was 6.125, 12.25, 24.5, 49 and 98 mg L^{-1}. For the control samples, without any added Acarbose, 7 mL of water was added to 350 mL normal and acidified custards and mixed.

2.2.3 Sensory assessment

An untrained panel of 30 assessors rated, in order, the attributes: taste intensity, creaminess and thickness on non-structured line-scales. An untrained panel was used because of interest in a consumer population and in the variation in perceived quality, although it is accepted that variability among assessors is likely to be higher than with a trained panel. The assessors were presented with 5 mL of custard sample in a plastic cup with a plastic spoon. Three digit codes were used to label the samples and they were presented in a random order. The assessors took the sample into their mouth, assessed it and spat it out into an empty cup. No conditions or time restraints were imposed. The assessors themselves measured the temperature of the spat out boli and the samples were then collected for analyses. All samples were tested by all assessors and replications were made on another day giving a full-factorial design containing 720 samples.

Full factorial design:

2 different pHs (normal: pH 6.3 and acidified custard: pH 4.2)

6 Acarbose levels (0, 6.125, 12.25, 24.5, 49 and 98 mg L^{-1})

30 assessors

2 times (on two different days)

2.2.4 Chemical analysis

The boli were stored at -40 °C in order to precipitate the mucins. The samples were then thawed at room temperature and pH, vanillin concentration and α-amylase activity were measured. Vanillin concentration was determined by reversed phase HPLC measuring the absorption at 277 nm. A Hypersil BDS–Phenyl, 25 *x* 4.6mm^2 5 µm column (Hewlett Packard, Waldbronn, Germany) was used with the mobile phase containing sodium-dihydrogenhosphate monohydrate, phosphoric acid and acetonitrile. The flow rate was 1.0 mL min^{-1} and vanillin eluted at a retention time of 7.9 min. The vanillin concentration was used to calculate the dilution of the custard with saliva.

Alpha-amylase activity was determined on thawed boli using a standard assay kit from Salimetricsr (State College, PA, USA). The buffered substrate, when broken down by

α-amylase, yields 2-chloro-p-nitrophenol and this was spectrophotometrically measured by its absorbance at 405 nm. The analysis was done under standard in vitro conditions at pH 7.0 and 37 °C, according to the manufacturer's recommendations. This activity, measured under standard conditions was expressed as units of activity per mL of bolus and, because these conditions might be different from the in situ conditions, the values are referred to as 'potential' activity. The pH strongly affects the α-amylase activity (Evans et al., 1986; Merritt et al., 1977; Wakim et al., 1969) and the in situ activity was calculated, at the bolus pH, from its potential activity at pH 7.0, according to the literature pH dependency of α-amylase activity (Wakim et al., 1969).

2.2.5 Mechanical measurements

The thickness of the normal and acidified custard was measured instrumentally by back extrusion. The measurements were carried out using a Texture Analyser (with a 5 kg load cell; Stable Micro Systems Ltd., Surrey, UK). The custard, 100 mL, was put in a cylinder (50 mm internal diameter) and a circular probe (45 mm diameter and 6 mm high) was lowered into the sample so that the probe was covered by the custard, equilibrated at 30 °C. During the measurement the probe was pushed through the custard 1 cm at a constant speed of 2 mm s^{-1} and the force–time curve was recorded at 200 points s^{-1}.

2.2.6 Statistical analysis

The data from the normal custards and the acidified custards were treated as two individual data sets since the two products had initially different textures. Pearson correlation coefficients were calculated to assess the association among the sensory attributes on the data from both days (sessions). The other analyses were done on the data from only one day. The effect of assessor (n = 30) and Acarbose (n = 6) on thickness, taste intensity, creaminess, potential α-amylase, dilution factor, temperature and pH was calculated using a 2-factor (with replication) analysis of variance. Linear regression analysis was applied to investigate the impact of potential α-amylase, dilution factor, temperature and pH on the sensory attributes eliminating assessor effects. Prior to analysis the data for potential α-

amylase and dilution factor were logarithmically transformed to obtain more symmetric data distributions.

2.3 Results

All sensory attributes were scored, on average, at the middle of the scale. The relationships between the sensory attributes were highly significant although little of the variance in any one attribute was explained by that in another. For the normal custard (Figure 2.1a), taste intensity related more to creaminess (r = 0.69) than to thickness (r = 0.50) and creaminess was strongly related to thickness (r = 0.77). For the acidified custard (Figure 2.1b), creaminess was more correlated to thickness (r = 0.67) than to taste intensity (r = 0.52) and taste intensity was more correlated to thickness (r = 0.58).

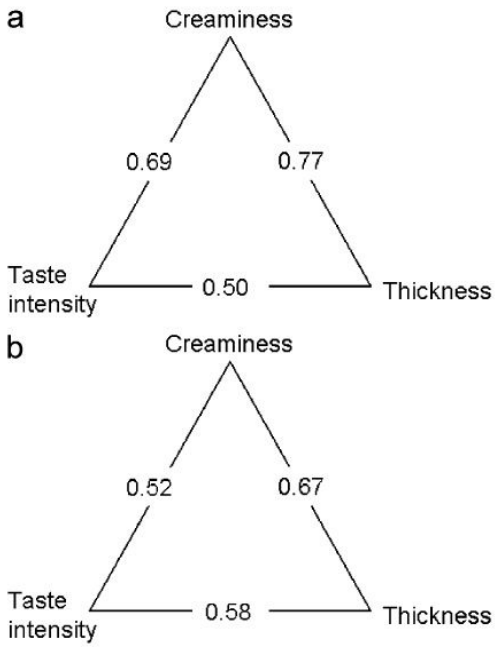

Figure 2.1 – Correlations of the sensory attributes for the normal custards (a) and for acidified custards (b)

The mean values of the sensory response showed the differences between normal and acidified custards and also an effect of added Acarbose (Table 2.1).

Table 2.1 – The effect of different Acarbose levels and acidification on assessors' ratings of taste intensity, creaminess and thickness[a]

Acarbose (mg L^{-1})	Taste (0-100)		Creaminess (0-100)		Thickness (0-100)	
	Normal	Acidified	Normal	Acidified	Normal	Acidified
0	51.4	61.4	50.5	50.5	47.3	61.6
6.125	52.6	62.3	51.6	53.5	49.4	61.9
12.25	51.3	61.1	51.7	55.6	47.5	63.3
24.5	52.5	59.3	50.6	51.7	48.1	59.4
49	52.1	59.5	51.8	53.1	48.7	61.7
98	50.4	60.6	57.6	52.5	58.3	62.6
Overall mean	51.7	60.7	52.3	52.8	49.9	61.8
SED	2.14	2.35	2.13	1.96	2.50	2.39

[a] Values are the means and standard error of the difference (SED) of 30 assessors and 2 replicates for each Acarbose level.

Addition of up to 98 mg L^{-1} Acarbose of custard had no systematic effect on taste intensity, neither in the normal nor the acidified custards. Acidification made the taste about 17 % more intense than without acidification. Addition of 98 mg L^{-1} Acarbose caused a 23 % increase of the perceived thickness and a 14 % increase in perceived creaminess, of normal custard, but had no effect on the thickness and creaminess of the acidified custard. Acidified custards were perceived consistently thicker than normal custards and, overall, thickness was rated 62 for acidified custard and 50 for normal custard. Back extrusion force measurements showed that the acidified custard with no added Acarbose was about 25 % thicker than the nonacidified one (Figure 2.2).

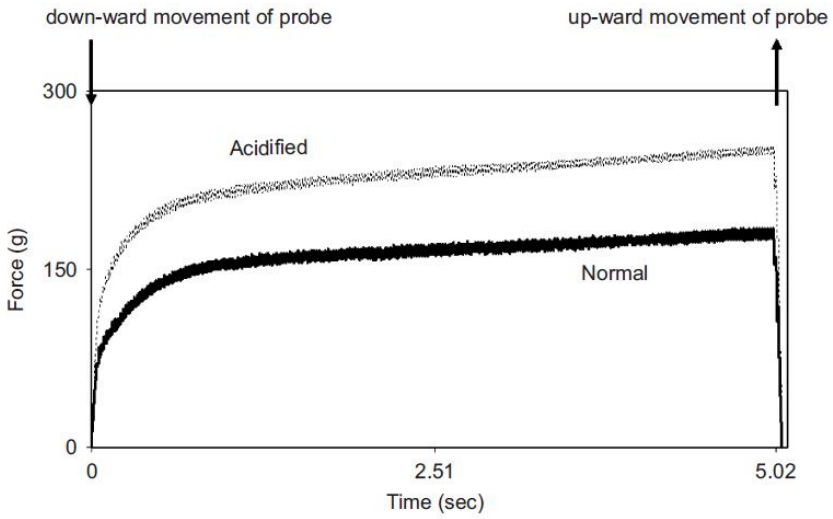

Figure 2.2 – Force-time curves for back extrusion test of normal and acidified custard

Mean values from the chemical and physical analyses (Table 2.2) showed that for the normal custard there was an asymptotic decrease in potential α-amylase activity with increasing Acarbose concentration. In normal custard, the addition of 98 mg Acarbose L^{-1} more than halved the α-amylase activity in the bolus compared with normal custard without Acarbose. A steep decrease in activity could first be seen at 12.25 mg mL^{-1} and there was little further decrease in activity with further increase in Acarbose concentration. The bolus from the acidified sample had a 77 % higher potential α-amylase activity, a 5 % higher dilution and a 35 % lower pH than the normal custard. Apart from the effect of Acarbose concentration on the α-amylase activity, up to 98 mg Acarbose per litre had no systematic effect on the dilution, temperature or pH of the bolus.

In table 2.3, the variation among assessors on the sensory ratings for normal custard samples without Acarbose is shown as mean and minimum and maximum values. The range of values was especially large for the sensory ratings, for the potential α-amylase activity and also, to a lesser extent, for the dilution factor. The addition of Acarbose did not affect the variation (Table 2.4) among assessors in sensory ratings, temperature or pH but it increased, by 40–50 %, the variation in potential α-amylase activity and dilution factor.

Table 2.2 – The effect of Acarbose and acidification on α-amylase activity, dilution factor, temperature and pH of the bolus[a]

	Potential α-amylase activity (U mL^{-1})		Dilution factor		Temperature (°C)		pH	
Acarbose (mg L^{-1})	Normal	Acidified	Normal	Acidified	Normal	Acidified	Normal	Acidified
0	54.8	89.8	2.48	2.46	30.5	30.1	6.82	4.99
6.125	54.5	44.2	2.41	2.28	30.5	30.1	6.94	5.16
12.25	32.9	55.8	1.97	2.30	30.6	30.2	6.94	5.11
24.5	29.1	67.6	1.85	2.26	30.4	30.2	6.96	5.19
49	26.2	75.9	1.89	2.39	30.6	30.2	6.92	5.15
98	24.8	60.4	2.34	2.04	30.4	30.0	6.92	5.18
Overall Mean	37.1	65.6	2.2	2.3	30.5	30.1	9.9	5.1
SED	5.03	9.69	0.12	0.07	0.16	0.18	0.04	0.04

[a] Values are the mean and standard error of the difference (SED) of 30 assessors for each Acarbose level

Table 2.3 – Variation among assessors' ratings for thickness, taste intensity and creaminess and among the potential α-amylase activity, dilution factor, temperature and pH in the bolus[a]

	Mean	Minimum	Maximum
Thickness rating (0-100)	47.3	3.5	83.8
Taste rating (0-100)	51.4	0.6	95.9
Creaminess rating (0-100)	50.5	0.8	97.0
Potenital α-amylase activity (U mL^{-1})	54.8	0.7	144.0
Dilution factor (ratio)	2.5	1.7	2.6
Temperature (°C)	30.5	27.7	32.3
pH	6.8	6.6	7.0

[a] Means and extreme values for 30 assessors for normal custard without added Acarbose

Table 2.4 – Coefficient of variation (standard deviation x 100 / mean) among assessors for sensory ratings and composition of expelled boli from the normal custards with 0 and 98 mg L^{-1} Acarbose

	Coefficient of variation (%)	
	0 mg L^{-1} Acarbose	98 mg L^{-1} Acarbose
Thickness rating (0-100)	35	31
Taste rating (0-100)	37	43
Creaminess rating (0-100)	33	34
Potenital α-amylase activity (U mL^{-1})	74	105
Dilution factor (ratio)	25	38
Temperature (°C)	4	5
pH	2	4

Analysis of variance was done on individual assessor means of the 2 replicates and for comparison with objective data measured only once. This showed that, for the normal custard, variation in assessors and Acarbose accounted for 58 % of the variance in thickness and 77 % of the variance in creaminess. Linear regression analysis for normal custard

showed that, after having fitted assessor effects, variations in dilution, pH, temperature and α–amylase explained only 11 % of the variation in thickness and only 7 % in creaminess. For the acidified custard the majority of the variation was due to differences between assessors. However, there was a significant Acarbose effect for thickness and creaminess under normal conditions and for potential α-amylase activity, dilution and pH under both normal and acidified conditions (Table 2.5). There was a significant effect of potential α-amylase activity and dilution on thickness and creaminess under normal conditions after removing the effect of Acarbose. The precision of the regression coefficient was low, based on the standard errors for these coefficients.

2.4. Discussion

In this study a simplified sensory profile was used. More complex profiles often include 'melting' (de Cock & Vanhemelrijk, 1995; de Wijk, van Gemert, Terpstra, & Wilkinson, 2003; Elmore, Heymann, Johnson, & Hewett, 1999) defined as 'the (rate of) thinning of food in the mouth', which is presumed to be caused by α-amylase degradation of starch (de Wijk et al., 2004). However, ratings of 'melting' were negatively correlated to the decrease in shear thinning between 30 and 60 s (de Wijk et al., 2003) and may have been confused semantically with 'thin'. 'Creaminess' is a well recognised attribute among consumers (Richardson-Harman et al., 2000). Creaminess ratings by expert panels have been found to be related to 'thickness' (Elmore et al., 1999; Kokini, 1987) but also almost independent of 'thickness' ratings (de Wijk & Prinz, 2006). In this work creaminess was a complex sensation related to both thickness and taste. A high level of Acarbose, approximately 20 times the concentrations used in this study, decreased creaminess ratings by 50 % (de Wijk et al., 2004). However, the amylase activity was not measured and so Acarbose may have also had an indirect effect on creaminess.

The best way to measure the influence of composition of the bolus on sensory perception would be to measure what happens on the tongue surface. However no methods are available to do this although continuous monitoring of mouth–material interactions is being developed (Adams, Singleton, Juskaitis, & Wilson, 2007). Measurements on the boli showed that the effect of Acarbose in increasing perceived thickness and creaminess can be

Table 2.5 – The effect of assessor and Acarbose on sensory ratings and in-mouth variables determined by analysis of variance

Source	Degrees of freedom	Thickness[a]	Taste intensity[a]	Creaminess[a]	Potential α-amylase activity[a,b]	Dilution factor[a,b]	Temp[a]
Normal custard							
Assessor	29	1526^{***}	2286^{***}	2519^{***}	7.04^{***}	0.087^{***}	9.17^{***}
Acarbose	5	433^{*}	110	257^{*}	4.56^{***}	0.46^{***}	0.27
Error	145	167	139	112	0.17	0.027	0.39
Acidified custard							
Assessor	29	1975^{***}	2220^{***}	2718^{***}	8.70^{***}	0.235^{***}	10.70^{***}
Acarbose	5	138	96	87	2.05^{***}	0.126^{***}	0.28
Error	145	86	145	122	0.23	0.014	0.46

[a] Values are mean squares and significance for assessor and Acarbose effects: *** $P < 0.001$; * $0.01 < P < 0.05$, referring to an F-distribution for the F-ratio

[b] Potential α-amylase and dilution factor were logarithmically transformed prior to analysis

explained as Acarbose reduced the α-amylase activity by 50 %, thereby reducing thinning from starch breakdown.

Acidifying the custard with citric acid decreased its pH from 6.3 to 4.2 and that of the bolus from 7 to about 5 so little buffering action from the saliva had occurred in the mouth. In milk, the proteins precipitate as the pH approaches the effective pK, at around pH 5, and the micelles then aggregate to form a gel (Holt & Roginski, 2001). This may be the reason why acidification of custard increased viscosity and perceived thickness.

Increased viscosity lowers the release of flavour molecules (Cook, Hollowood, Linforth, & Taylor, 2003; Malone, Appelqvist, & Norton, 2003; Pangborn, Trabue, & Szczesniak, 1973; Weel et al., 2002). Despite this, the acidified samples were perceived as having more taste than the normal ones and, as we did not ask specifically for a flavour, this was most likely due to the more intense acid taste. In the bolus, after dilution 2.3 times by saliva incorporation, the citric acid would be about 15 mM, still above the sensory threshold. The more intense taste may also have been due to an interaction of the different sensory information in perception.

The α-amylase activity in situ was estimated from activity determined in vitro and the pH, temperature and dilution in the bolus. The pH has a strong effect on α-amylase activity with an optimum activity around pH 7 (Evans et al., 1986; Merritt & Karn, 1977; Wakim et al., 1969), which is the pH in the in vitro assay. In the acidified condition, in which the bolus pH is around 5, the α-amylase activity would be decreased by 46 % (Merritt & Karn, 1977; Wakim et al., 1969). Surprisingly, our results showed that the estimated in situ α-amylase activities were similar in the boli from normal and acidified custards (Table 2.6). Therefore, the increased salivation and the amount of the α-amylase would be expected to increase α-amylase activity. However, the activity was not affected due to the lower pH of the bolus. Considering the variation among subjects, more than half of them had, for the acidified custards, none or only partial α-amylase activity when the low bolus pH was taken into account.

Acidification of custard to a final 30 mM citric acid stimulated a 5 % (or 0.1 mL) greater dilution by saliva compared with normal custard. This dilution approximates to the increase in parotid salivary flow of 0.2 mL min^{-1} which is produced with 5 mM citric acid

in water (Froehlich et al., 1987). It is likely therefore that most of the citric acid in the thick custard did not stimulate salivation. This is comparable to the lower sensory intensity of tastants in foods than in water (Cook et al., 2003; Malone et al., 2003; Pangborn et al., 1973).

Table 2.6 – The potential α-amylase activity is that activity measured in the boli under standard conditions[a]

	Alpha-amylase activity (U mL^{-1})	
	Normal	Acidified
Potential	37.1	65.6
In situ	35.9	39.4

[a] The in-situ activity is calculated from potential activity modified by the in-mouth variations in pH and temperature. Values are averaged from all Acarbose levels and assessors

Understanding the causes of the variation among people will be important for product development and targeting. In this work, salivary α-amylase activity varied 200-fold among the 30 subjects, although up to an 800-fold variation has been recorded (Kivela et al., 1997). Furthermore salivary pH can vary between 5.8 and 8.0 and its buffering capacity can vary more than 6-fold (Christensen et al., 1987). Reducing the α-amylase activity by adding Acarbose would be expected to reduce the variation among subjects resulting in more consistent ratings; however, this was not found to be the case. Previous work had not investigated this possibility. Even if more Acarbose would have been added, it would probably not have reduced the variation among subjects since Acarbose is a competitive inhibitor with most of the decrease in α-amylase activity at low concentrations.

Linear correlation analysis showed that very little of the variation in attribute ratings was accounted for by the measured chemical variables. Even after normalising the data among assessors, chemical variation still only accounted for 11 % for thickness and 7 % for creaminess. The question remains: What causes the rest of the variation? In this study the assessment time was not restricted in order to simulate normal eating conditions

for each individual. With custard, this residence time in the mouth is only a few seconds and it is not certain that this time is large enough for the α-amylase to break down the starch sufficiently to reduce viscosity. The viscosity of saliva has been shown to change depending on the type of stimulus, for example by drugs which may increase mucin concentration (Aps & Martens, 2005). Variation in mixing of the custard with saliva would however account for some of the variation in starch breakdown. Some people mix the custard better than others but no data are available on this for normal eating. In an experimental situation, moving the tongue up and down twice over 2 s showed that the mixing varied by about 250 % among assessors (Prinz, Janssen, & de Wijk, 2007). Because of the low oral degradation of starch in semi-solid foods, lowering their glycaemia index is unlikely to affect cognitive perception of texture.

2.5. Conclusions

In a starch-based dairy product, α-amylase activity was lowered and thickness and creaminess perception were increased by the addition of an amylase inhibitor. Addition of Acarbose reduced saliva α-amylase activity by more than half and increased perceived thickness by 25 %. Acidification, lowering the pH of the product from 6.3 to 4.2, increased the perceived and mechanical thickness. However it did not reduce the in situ α-amylase activity in the bolus because the saliva stimulated was also more concentrated in α-amylase. The composition of the stimulated saliva varied widely among subjects. Assessor effects on the sensory ratings were highly significant. The majority of the variation in sensory in both the acidified and the normal custard was unrelated to variation in α-amylase activity among assessors. Formulating products to decrease oral α-amylase activity by half would therefore not guarantee an increase in perceived thickness of starch-based foods.

2.6. Acknowledgements

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Chapter 3

Effects of different tastants on parotid saliva flow and composition

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Abstract

Saliva from parotid glands plays a role in taste perception. Parotid saliva is also stimulated by tastants. The aim of this work is to investigate the effects of different tastants on the parotid salivary response in six subjects. Five tastants were given in different concentrations in solution and held in the mouth for 10 s. The flow rate, protein concentration, and pH of secreted parotid saliva were monitored continuously for 5 min. Stimulation by tastants on flow rate response consists of an immediate rise in flow followed by a plateau and a rapid return to prestimulus flow. Response of pH results in a slower increase while protein concentration consists in a slower decrease, both followed by a return to prestimulus levels in about 4 min. From a resting flow rate of about 140 μL/min, an increase in flow rate to 370 μL/min was caused by stimulation for 10 s with 10 mL of solutions of 0.01 M citric acid, 0.13 M $MgSO_4$, 0.25 M monosodium glutamate, 0.5 M NaCl, or 0.5 M sucrose. Comparisons of the different tastants showed that the pH of stimulated parotid saliva increased linearly ($r = 0.9$), irrespective of the nature of the tastant. Protein concentration decreased ($r = -0.45$) and protein amount increases ($r = 0.58$) with increase in flow rate for all tastants. Corrected for the effects of flow rate, protein amount depended on the nature of the tastant with the greatest secretion after stimulation by citric acid. Flow rate was largely responsible for pH but tastant appears to play an additional role with flow rate on protein secretion.

3.1 Introduction

Saliva plays an important role in teeth maintenance by its antimicrobial action and also in digestion and food perception (Mese and Matsuo 2007). During these two last actions, saliva is predominantly released from the parotid glands. During eating, the proportion of parotid saliva in whole saliva can increase from 0 % to more than 50 % (Humphrey and Williamson 2001). Such an increase, due to stimulation by mastication and/or taste compounds, has been called the parotid salivary reflex (Chauncey and Shannon 1960). Consequently, parotid saliva participates largely in bolus formation and digestion, e.g., by the contribution of α-amylase in starch hydrolysis. In addition, parotid saliva contributes to taste perception. The large amount of bicarbonate ions in parotid saliva has a buffering action on acids, thus modifying sourness perception (Christensen et al. 1987; Lugaz et al. 2005). Moreover, bicarbonate concentration increases with flow rate and since flow rate tends to increase with acidity, the parotid saliva reflex promotes the protection of the oral medium against acidification. This implies that salivary response is adaptive, since the nature of the response is modulated by the "harmful" nature of the stimulus. This is supported by earlier finding showing that whole saliva proteome can be modified by the nature of the tastant with an overexpression of proteins involved in inflammatory response after stimulation by sour, bitter, and umami tastants but not after sweet (Neyraud et al. 2006). Others reported a possible specific response of parotid saliva to tastant by a different protein pattern expression (Dawes 1984) or an increase of α-amylase concentration after drinking sugar solution but not after sham drinking, suggesting a metabolic adaptation of the parotid glands and their specific participation in the digestion and regulation of appetite (Harthoorn et al. 2008).

Studying parotid saliva characteristics in response to a stimulus is complex. It was shown that characteristics like protein concentration or pH are linked to flow rate especially when stimulated by mastication (Neyraud et al. 2009). When collected with a Lashley cup after stimulation and analyzed in vitro, parotid saliva does not resemble saliva at the exit of the parotid duct. This is due to the delay existing to reach the exit of the collecting tube from the exit of the parotid duct. The delay depends of the volume of the tubing and the flow rate. Batch sampling is also not recommended for chemical reasons, for example, the

diminution of the buffer capacity due to CO_2 production from bicarbonate ions at the contact of the air. In addition, this batch-wise analysis of saliva does not allow characterization with high time resolution, which is desirable for the study of adaptive response to stimuli. We have developed a system able to collect parotid saliva from the exit of the parotid duct with a Lashley cup that continuously measures flow rate, pH, and protein concentration by absorbance (A280). This system synchronizes these continuous measures in time as if they were measured at the exit of the duct (Neyraud et al. 2009).

The aim of this work is to study continuous time-release profiles of parotid saliva characteristics in response to different tastant stimulations in order to establish relationships between pH and protein concentration as a function of flow rate.

3.2 Material and Methods

3.2.1 Subjects and Protocol

Three male and three female subjects, aged 22 to 39 years, non-smokers and of good general health participated in three morning sessions of 2 h each. While subjects were sitting upright, a Lashley cup was fitted over the exit of the duct of the right parotid gland. Then, subjects chewed a piece of parafilm until the collection system (820 µL) was filled with saliva. Each session started with a rest of 5 min. Then, a 15 mL medicine cup of distilled water was presented to the subject who was instructed to sip the solution during 10 s in a uniform fashion, to spit it out, and to have a rest of 5 min before the next solution. During the first session, increasing concentrations of citric acid in distilled water were presented (0.25, 0.5, 1, 2.5, 5, 10, 30, 75, 150, and 300 mM). Following the same protocol, the second session consisted of tasting sucrose solutions (0.1, 0.2, 0.5, 1.5, and 2 M). After a break of at least 15 min during which the subject rinsed his mouth with water, NaCl solutions (0.05, 0.1, 0.2, 0.3, and 0.5 M) were tasted. Finally, during a third session, solutions of monosodium glutamate (MSG; 0.12, 0.25, 0.5, 0.75, and 1 M) and $MgSO_4$ (0.25, 0.5, 0.75, 1, and 1.25 M) were presented. The first solution tasted at the beginning of each series was distilled water. In sucrose, NaCl, MSG, and $MgSO_4$ sessions, the last stimulus used was always 10 mL of a 75 mM citric acid solution. The collection system

was then flushed by chewing a piece of parafilm for 5 min to acquire measures from the saliva that was still in the system after the last stimulation.

The protocol was carried out in accordance with the guidelines of Ethical Committee of Wageningen University. The subjects reported no discomfort during the testing. All subjects gave informed consent.

3.2.2 Continuous Recording of Parotid Saliva

The system, used to collect and measure parotid saliva characteristics, was described in detail elsewhere (Neyraud et al. 2009). Therefore, here, we just give a brief description.

Parotid saliva was collected from the orifice of the Stensen's duct using a Lashley cup connected by 0.4 m of Tygon tube (internal diameter of 0.5 mm) to a flow meter (tubing volume between Lashley cup and flow meter 242 μL), an absorbance cell (tubing volume 261 μL), and a pH probe (tubing volume 430 μL). The flow was recorded with an ASL 1430-16 liquid mass flow meter (Sensirion, Stafa, Switzerland). Absorbance (A280) was determined through a 1.5 mm light path with an internal volume of 20 μL using a deuterium light source DH-2000-BAL (Ocean Optics, The Netherlands). From stimulated and nonstimulated saliva, protein concentration (Bradford protein assay Quick Start™; Bio-Rad, The Netherlands) was linearly related ($R = 0.87$, $p < 0.01$) to absorbance: protein concentration (g/L) = 0.35 (A280) + 0.49. The pH was measured with a FTPH 2 S probe (Lazar Research Laboratories, California) coupled to an A to D converter (PT-104, Pico Technology, UK).

Flow rate, A280, and pH values were sampled synchronously at 3.125 Hz and were assigned to release times into the Lashley cup that were calculated from cumulative flows and the volumes between the Lashley cup and the absorbance cell and the pH probe, respectively. All sampling, calibration, and calculations were performed continuously by a Delphi-based (Borland Software Corp., Cupertino, CA, USA) computer program (Neyraud et al. 2009).

3.2.3 Variables Selected

Stimulations by tastants yielded similar time–response curves for flow rate, pH, and A280 nm (Fig. 1). Three variables were defined to characterize these curves quantitatively in terms of flow rate response and event time: The peak of flow (F_{peak}) consisting of the average of the five maximum values following the instantaneous increase of flow rate after stimulation and its corresponding time ($T–F_{peak}$), the accumulated flow during the first minute after stimulation ($F_{60\,s}$) corresponding to the volume secreted during the first minute after stimulation, and the total flow (F_{tot}) corresponding to the volume of fluid secreted after stimulation until a return to a baseline level (before stimulation) and its corresponding time ($T\text{-}F_{tot}$). Measured pH was expressed as the maximum pH value after stimulation (pH_{max}). Two variables related to proteins were the protein concentration (P_{conc}) corresponding to the average concentration of protein secreted during the 5 min after stimulation and the total protein amount (P_{tot}) which corresponds to the instantaneous concentration in protein multiplied by the corresponding flow rate.

3.2.4 Data Analysis

Data analysis consisted of two stages:

(a) Since the main interest is in the effects of flow rates and tastants on saliva composition, the first stage consisted of testing whether the used method of manipulating stimulus concentration and tastant indeed affected flow rates significantly. Hence, effects on flow rate were tested by analysis of variance (ANOVA) for the variables tastant (fixed factor; five categories), concentration (fixed factor; ten categories for citric acid, five categories for sucrose, MSG, $MgSO_4$, and NaCl), and subjects (random factor; six categories). The test included all main effects and two-way interactions.

(b) Taking into account the expected effects of flow rate after stimulation on salivary pH, average protein concentration during the first 5 min after stimulation (P_{conc}, milligrams per millilitre), and total amount of protein released during the first 5 min after stimulation (P_{tot}, milligrams), the modulating effects of tastant on pH,

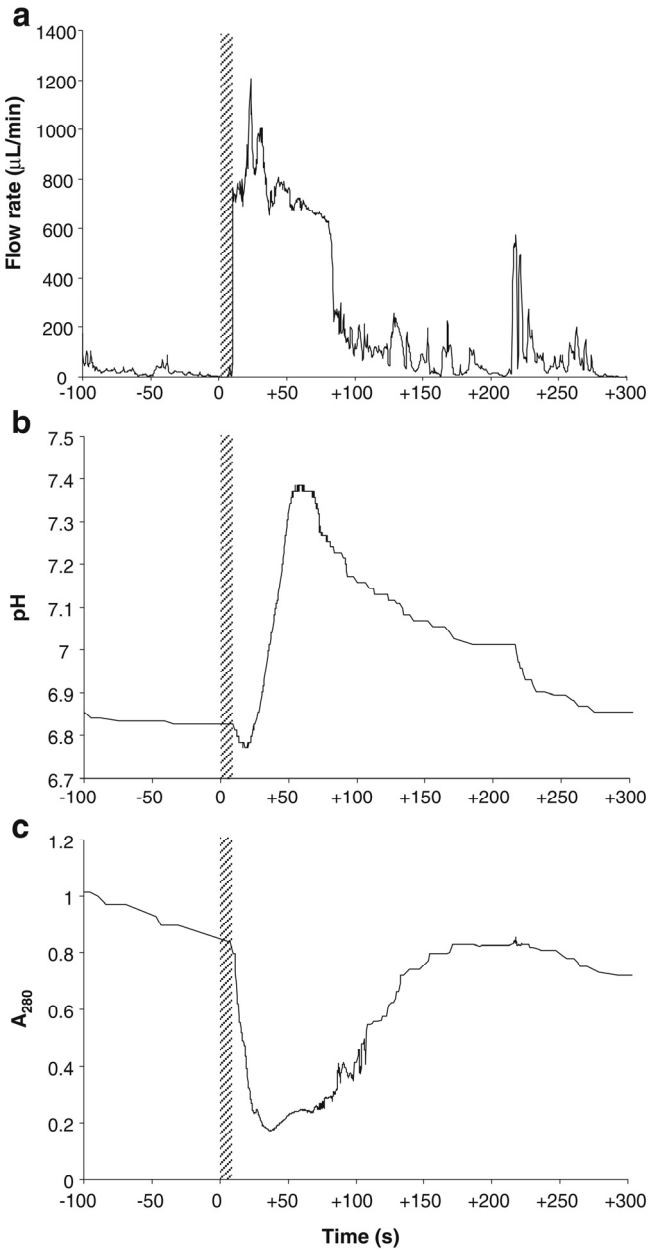

Figure 3.1 – Continuous parotid saliva flow (a), pH (b), and absorbance at 280 nm (c) of one subject in response to 10 mL of 30 mM citric acid placed in the mouth for 10 s (at hatched lines)

P_{conc}, and P_{tot} were tested by ANOVA, including flow rate as a covariate, tastant as a fixed factor (five categories), and subjects as a random factor (six categories).

Duncan's multiple range statistic was used for post hoc analysis of tastant effects, performed on data from which trends due to flow rate were removed. This prevents spurious effects introduced by an inhomogeneous distribution of tastants over flow rates.

3.3 Results

3.3.1 Analysis of the Continuous Response Curves

Continuous flow rate, pH, and A280 curves after stimulation with 30 mM citric acid for one subject are presented in figure 3.1. Qualitative description of the response can be done as follows: The stimulus induced an instantaneous increase in flow rate from less than 10 µL/min to a maximum value (about 1,200 µL/min) after about 30 s. The flow rate remained at 600µL/min for a further 50 s before decreasing abruptly and reaching the resting level after a further 100 s (Figure 3.1a). A similar evolution was seen for pH. The pH increased from a resting value of 6.85 to a maximum value of 7.4 at 60 s before decreasing slowly to the resting level (Figure 3.1b). The A280 pattern was the mirror image of pH with a decrease following the stimulus following by a slow increase (Figure 3.1c).

A delay can be observed for reaching either the peak of pH or A280. This can be explained by the diffusion of compounds within the system. For other subjects and tastants, the response curves were following the same trend.

3.3.2 Tastant Effects on Flow Rate

F_{peak} value is always higher than the corresponding $F_{60\,s}$ or F_{tot}, except for citric acid concentrations over 10 mM. For all tastant, $F_{60\,s}$ was similar to F_{tot} except for citric acid concentration over 10 mM (Figure 3.2). No significant effects of the tastant nature on the three flow measures were found on water and 75 mM citric acid stimuli, used at the beginning and at the end of each protocol, respectively.

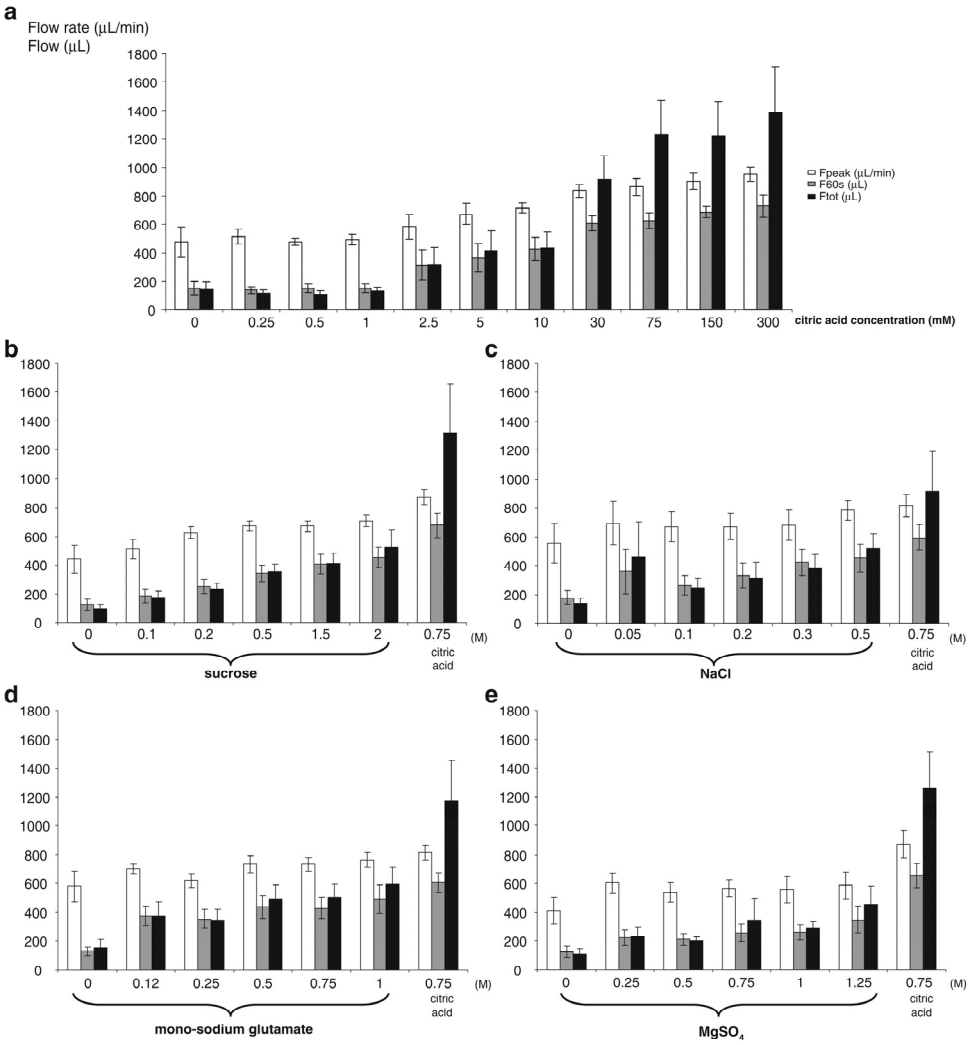

Figure 3.2 – Average maximum flow rate peak in *white* (microliters per minute), flow during 1 min in *gray* (microliters per minute) and total flow rate response in *black* (microliters) after stimulation by citric acid (a), NaCl (b), monosodium glutamate (d), and $MgSO_4$ (e). $N = 6 \pm$ SEM

There was no systematic salivary response for citric acid below 2.5 mM. Between 2.5 and 30 mM, flow rates increased and from 30 mM and onwards, flow measures remained at a plateau of 0.8 mL/min for the peak and 0.6 mL/min, for $F_{60\,s}$. F_{tot} reached a plateau (1.2 mL/min) above concentrations of 75 mM (Figure 3.2a).

For the other tastants tested, $F_{60\,s}$ and F_{tot} were always above the value obtained after stimulation with water and always below the values of the citric acid plateau suggesting that the used tastant solutions did not lead to a maximum response of the gland. The most consistent ordinal dose-response patterns were found for sucrose (Figure 3.2b) and MSG (Figure 3.2d). For NaCl (Figure 3.2c) and $MgSO_4$ (Figure 3.2e), dose–response patterns were less consistently ordinal.

ANOVA of flow rate results revealed significant effects of tastant [$F(4, 20) = 5.37$, $p < 0.01$], concentration [$F(15, 75) = 14.06$, $p < 0.001$], and significant interactions for tastant × subject [$F(20, 30) = 4.79$, $p < 0.001$] and concentration × subject [$F(75, 13{,}211) = 2.9$, $p < 0.05$]. Concentration effects can be described as steadily increasing flow rates for increasing concentrations and tastant effects as different intercept values for parallel concentration–flow rate functions of different tastants.

3.3.3 Temporal Flow Rate Response

There was no variation for the $T–F_{peak}$ between the different tastants and the concentration tested. The average time to reach the peak was usually 10 to 20 s after stimulation (Figure 3.3). $T\text{-}F_{tot}$ was increasing with the concentration of the taste compound. The longest $T\text{-}F_{tot}$ was reached with citric acid for concentration up to 75 mM (Figure 3.3a). Stimulations with NaCl do not show concentration effects on $T\text{-}F_{tot}$ (Figure 3.3c).

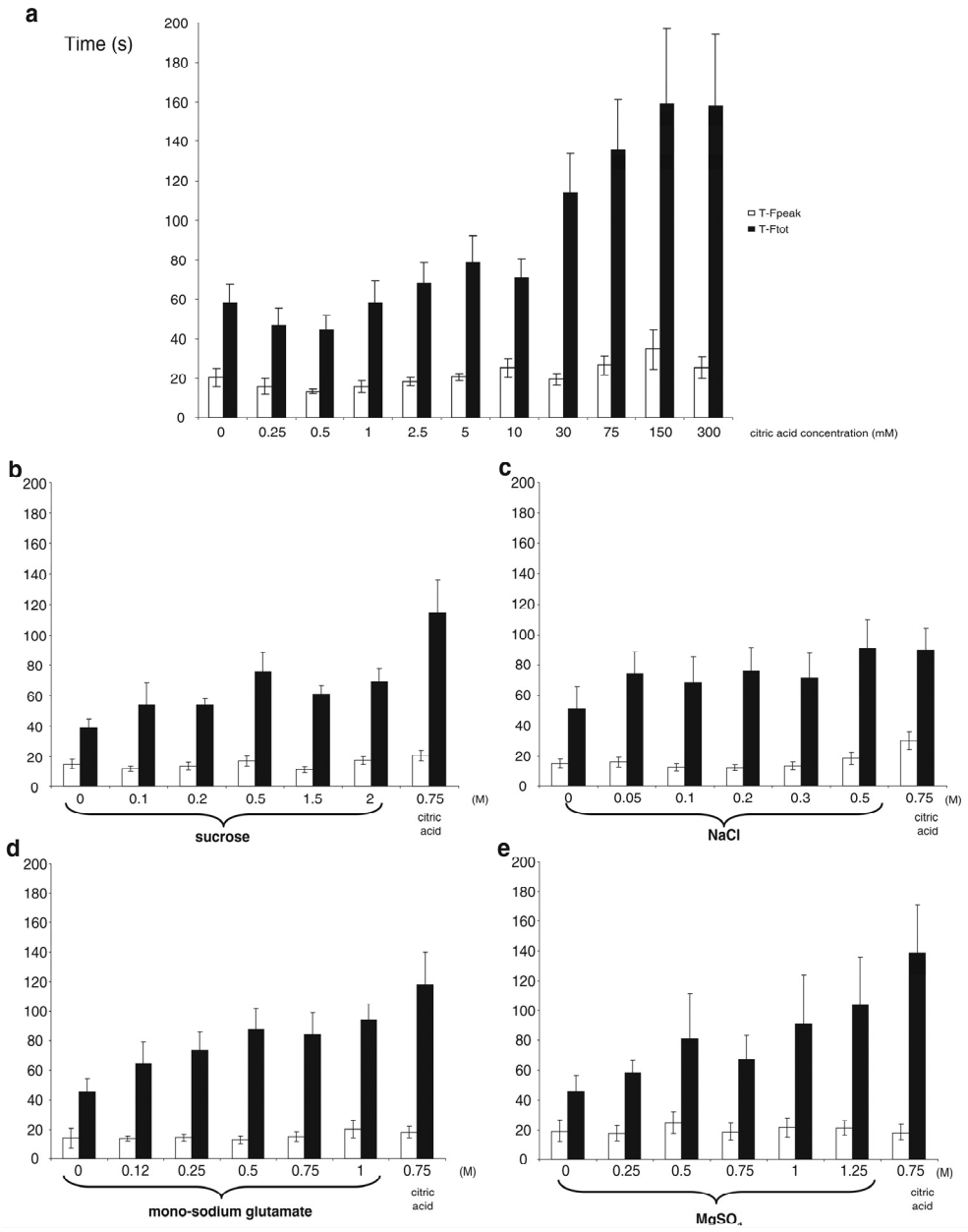

Figure 3.3 – Time to reach the flow rate peak (*white*) and total flow rate response (*black*) after stimulation by citric acid (a), sucrose (b), NaCl (c), monosodium glutamate (d), and $MgSO_4$ (e). $N = 6 \pm$ SEM

3.3.4 Relations pH and Flow Rate

The maximum pH after stimulation was significantly correlated ($r = 0.9$, $p < 0.00001$) to the flow rate over 1 min (Figure 3.4a) and increased linearly from 6.4 at 0.1 mL/min to 7.2 at 0.7 mL/ min. Considering all the tastants, the pH was similar for all tastants if flow rates were similar. However, there was a high variability in pH values at flow rates below 0.2 mL/min. ANOVA of pH results, with flow rate as a covariate, revealed no significant tastant effects [$F(4, 168) = 0.370$; $p = 0.83$].

3.3.5 Relations Protein and Flow Rate

Protein concentration (P_{conc}), averaged over the 5 min period after the stimulation (Figure 3.4b), shows a significant decrease ($R = 0.45$, $p < 0.01$) with flow rate ($F_{60\,s}$). ANOVA of Pconc results, with $F_{60\,s}$ as a covariate, revealed significant tastant effects [$F(4, 174) = 5.39$; $p < 0.001$]. Post hoc analysis of tastant effects on P_{conc} data revealed that citric acid group has a higher P_{conc} than a subgroup formed by $MgSO_4$, sucrose, and MSG while NaCl group is intermediate (Figure 3.5).

Similar results were found on the amount of protein (P_{tot}) secreted during the 5 min after stimulation which increases significantly ($R = 0.58$, $p < 0.001$) with $F_{60\,s}$ (Figure 3.4c). ANOVA of P_{tot} results, with $F_{60\,s}$ as a covariate, revealed significant tastant effects [$F(4, 175) = 4.55$; $p < 0.01$]. Post hoc analysis of tastant effects on P_{tot} data revealed that citric acid group has a higher P_{tot} than a subgroup formed by $MgSO_4$, sucrose, and MSG while NaCl group is intermediate (Figure 3.5).

3.4 Discussion

3.4.1 Taste and Flow Rate

Although different tastants may have different effects on flow rate (Speirs 1971; Hodson and Linden 2006), it is difficult to compare them in a straightforward design since different molecules do not have the same stimulation potential at specific concentrations. One option to study whether different tastants have the same physiological effect on parotid secretions

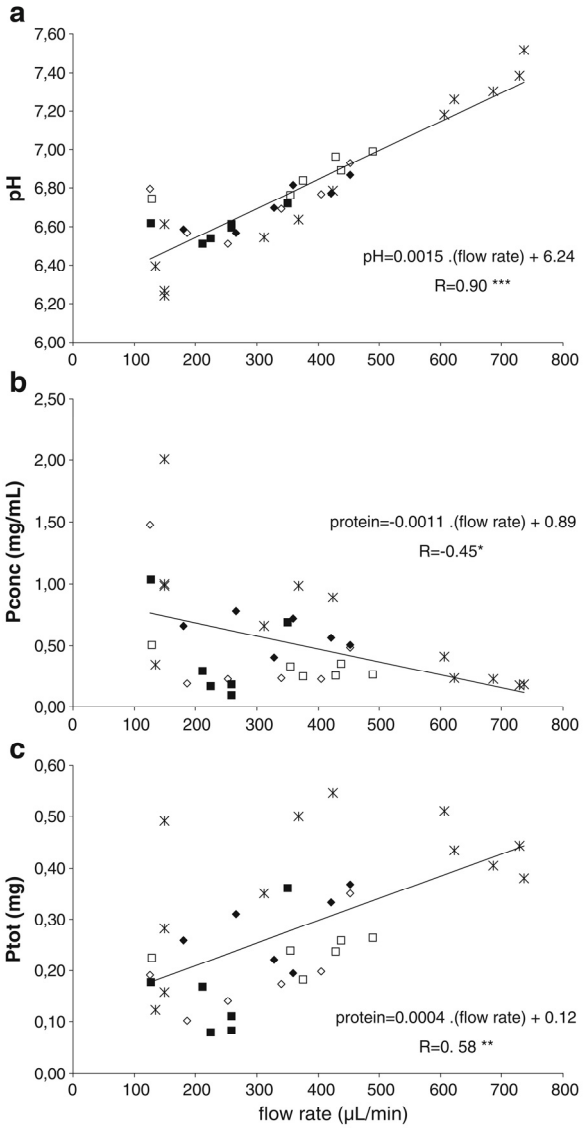

Figure 3.4 – Relationship between pH (a), protein concentration (b), total protein (c), and flow rate over the first minute after stimulation for the five different tastants. Values are the means of six subjects for citric acid (*star*), sucrose (*open diamond*), NaCl (*closed diamond*), monosodium glutamate (*open square*), and $MgSO_4$ (*closed square*). $N = 36$; * $p < 0.01$; ** $p < 0.001$; *** $p < 0.00001$

is to compare their effects when they give a similar response in terms of flow rate. Then, at this level, it is possible to compare other characteristics like pH or protein concentration.

In this study, we have compared three types of measurement of flow rate: the maximum value of the peak following the stimulation (F_{peak}), the flow rate during the first minute ($F_{60\,s}$), and the total response to the stimulation being the total flow from stimulation onset until the moment that flow returns to baseline (F_{tot}). The different taste molecules were selected in order to cause complete parotid flow response in less than 1 min.

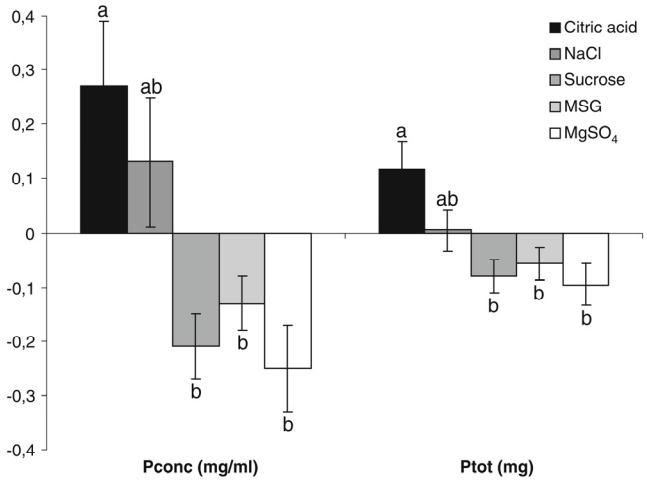

Figure 3.5 – Effect of tastant on protein concentration (P_{conc}) and total protein (P_{tot}) with flow rate as a covariate. Vertical bars show the standard error of the mean. Means lacking common letters differ significantly ($p < 0.001$ for P_{conc} and $p < 0.01$ for P_{tot})

Interestingly, time of the F_{tot} ($T\text{-}F_{tot}$) increased with tastant concentration whereas the Fpeak did not change. This was due to a persistence of the flow rate at plateau level. The duration of this plateau was related to the concentration of the tastant which could be due to a persistence of taste stimulation. Such persistence is difficult to stop since rinsing with distilled water is not relevant, this action by itself having a stimulating effect on parotid secretions.

Tastants were chosen in line with Hodson and Linden (2006) so that results could be compared with the results from this study. At a comparable concentration of about 75 mM citric acid, these authors observed peak values of 4 mL/min and a first-minute flow rate of 2.5 mL/min against 0.9 and 0.6 mL/min, respectively, in our study. These higher values can be probably due to an application of the stimulus for 30 s, in contrast with the 10 s presentations in the present study. Similar to this study, Hodson and Linden (2006) observed saturation plateaus from concentrations of 75 mM and up. Similar results were found at a concentration of 30 mM after the first minute of stimulation (Jensen-Kjeilen et al. 1987). Results concerning the other taste compounds are comparable to the ones obtained by Hodson and Linden (2006).

3.4.2 Taste and pH

Linking flow rate measurements to saliva pH at the exit of the parotid duct cannot be achieved by classical in vitro studies in human. When collected, the delay due to the length between the collecting tubing and the Lashley cup makes impossible to recombine a physical measurement (flow rate) to a chemical measurement (pH). Moreover, pH should be measured without contact with air to avoid loss of CO_2 from bicarbonate ions present in saliva (Bardow et al. 2000). In this study, it is the first time that we can link flow rate with pH measurements without loss of CO_2 after stimulation by tastants as if these were measured at the exit of the duct at high time resolution (3.125 Hz).

It is known that a negative relation exists between flow rate and pH. In parotid glands, when stimulated, HCO_3^- ions are generally assumed to be the main responsible molecules for buffer capacity (Tabak 2006). According to the two stages model, primary fluid secreted by salivary acinar cells is a plasma-like isotonic fluid rich in bicarbonate and NaCl (Melvin et al. 2005). When excreted, this solution is modified during passage through the duct system. Duct cells reabsorb Na^+ and Cl^-, secrete K^+, and either absorb or secrete HCO_3^- (Roussa 2001). These phenomena invoke a decrease of HCO_3^- concentration at the exit of the duct system at lower secretory rates when the system is more efficient (Park et al. 2002). Although these molecular mechanisms are well understood, it remains still difficult to predict pH of parotid saliva as a function of flow rate. In this work, we have

found a linear relation between pH (pH_{max}) after a stimulation and flow rate ($F_{60\,s}$) independently of tastant nature. In 1969, Dawes (1969) found, for constant flow of parotid saliva after stimulation with sour lemon, pH measures decreasing from 6.8 to 7.4 for flow rates from 0.25 to 1 mL/min, respectively. Although these results are in the range we found, the author did not establish a relationship between flow rate and pH. No important effects of the taste nature were found by the same author on the ionic composition of parotid saliva (Dawes 1984). Unfortunately, no indications about the flow rate of saliva during sample collections are available. Recently, we have found a similar relation between pH and flow rate after stimulation by chewing: pH = 0.0025 flow rate (μL/min) + 5.74 (Neyraud et al. 2009). This gives support to the fact that the relation between pH of parotid saliva does not depend of the nature of the stimulus but of the flow rate induced by this one.

3.4.3 Taste and Proteins

In this report, we did observe a decrease of protein concentration (P_{conc}) with increasing flow rate ($F_{60\,s}$). Interestingly, this protein decrease is spurious and only due to dilution since the total amount of protein (P_{tot}) released per time unit actually increases with flow rate. Although significant, these relationships are not clear since values given after stimulation by citric acid at high flow are influencing strongly the relationships and $F_{60\,s}$ larger than 500 μL were not achieved by other tastants.

Mechanisms for secretion of proteins in parotid glands are discussed in full detail in review articles (Turner and Sugiya 2002; Gorr et al. 2005) that generally support the notion that secretion is controlled by the autonomic nervous system. The sympathetic nervous system tends to evoke greater release of proteins and even higher when in synergy with the parasympathetic system (Proctor and Carpenter 2007). Some authors suggested that release of proteins in parotid saliva may depend on the nature of the stimulus. When compared to other tastants, citric acid stimulation results in a lower concentration in protein with a higher α- amylase activity (Froehlich et al. 1987). Unfortunately, the comparison was done for constant taste perception levels and not for constant flow rates. At a constant flow rate, Dawes (1984) had reported a higher protein concentration after stimulation by NaCl. Without flow rate effects, we did observe a higher amount of protein after citric acid

stimulation compared to sucrose, MSG, and $MgSO_4$ while NaCl evokes intermediate protein release. Increase of protein amount has already been reported by Dawes at a constant flow rate after long stimulation by NaCl. Also, an increase of α-amylase activity has been reported by Speirs et al. (1974) after application of ascorbic acid on the tongue and with stimulation of the sympathetic system. The authors suggested that such an oversecretion of protein could be due, in some way, to an increase of the ratio of sympathetic to parasympathetic stimulation of the gland causing a higher rate of protein secretion (Dawes 1984). A possible additive explanation could be the activation of the trigeminal lingual system in addition to the taste sensation after stimulation by acids conducting in sensations of irritation. Indeed, it has been reported that during perception of acid, the trigeminal free nerve ending are also stimulated (Lugaz et al. 2005). Recently, an overexpression of protein secretion in whole saliva has been found after stimulation by tastants with the strongest modification of the whole saliva proteome after stimulation by acid (Neyraud et al. 2006). The apparent oversecretion of protein in parotid saliva after stimulation by acid can be due to a synergic participation of the gustatory and trigeminal system.

3.5 Conclusion

This is the first time that flow rate, pH, and protein concentration and amount of saliva from parotid glands can be calculated as it were assessed at the exit of the parotid duct after stimulation by tastants. The linear relationship between flow rate and pH was established allowing the calculation of pH from flow rate. For protein concentration and total protein amount after stimulation, this relation is not clear. However, after correcting for flow rate effects, protein concentration and protein amount are significantly higher for stimulation by citric acid than for stimulation by sucrose, $MgSO_4$, and MSG.

3.6 Acknowledgments

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Chapter 4

Individually modified saliva delivery changes the perceived intensity of saltiness and sourness

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Abstract

Individuals vary largely in their salivary flow and composition and, given the importance of saliva on perception of taste, this might influence how the tastant stimuli are perceived. We therefore hypothesise that altering the individual salivary flow rates has an impact on the perceived taste intensity. In this study we investigated the role of saliva amount on perceived taste intensity by excluding parotid saliva and adding artificial saliva close to the parotid duct at pre-set flow rates. Significant decreases in perception with increasing salivary flow rates were observed for citric acid and sodium chloride. This can partially be explained by a dilution effect which is in line with previous studies on detectable concentration differences. However, since the bitterness and sweetness remained unaffected by the salivary flow conditions and the dilution effect was comparable to that of saltiness, further explanation is needed. Furthermore, we investigated if the suppression of taste intensity in binary mixtures (taste-taste interactions) could possibly be caused by the increased salivary flow rate induced by an additional taste attribute. The results show however that suppression of taste intensity in binary mixtures was not affected by the rate of salivation. This was more likely to be explained by psychophysics.

4.1 Introduction

The role of saliva in food perception has been studied extensively (Bonnans and Noble 1995; Christensen et al. 1987; Delwiche and O'Mahony 1996; Engelen et al. 2003; Froehlich et al. 1987; Heinzerling et al. 2008; Lugaz et al. 2005; Matsuo 2000; Norris et al. 1984; Speirs 1971). Saliva from various salivary glands contributes to the bolus formation, of which the parotid glands contribute to more than half of the total salivary volume upon stimulation (Mese and Matsuo 2007; Pedersen et al. 2002; Shannon 1962; Van Nieuw Amerongen et al. 2004). The salivary flow secreted upon stimulation enables transport of taste molecules to the taste bud (Matsuo 2000; Van Nieuw Amerongen et al. 2004). The composition of saliva is important for taste perception, i.e. the neutral pH of saliva along with its buffering action is of importance for the perception of sour stimuli (Christensen et al. 1987; Norris et al. 1984). Sodium salts present in saliva determine the level at which salt can be tasted in a product (Delwiche and O'Mahony 1996; Matsuo 2000; Spielman 1990). Furthermore, salivary enzymes start the process of digestion and can hereby influence the texture and taste perception by changing the viscosity of the food (Heinzerling et al. 2008). The amount of saliva secreted depends on the type and concentration of taste stimuli perceived (Dawes and Watanabe 1987; Froehlich et al. 1987; Hodson and Linden 2006; Neyraud et al. 2009; Speirs 1971). In a previous study, we have shown that the composition of saliva depends more on the flow rate than on the type of stimulus. However, the protein concentration varies between different types of tastant stimuli, independent of the flow rate (Neyraud et al. 2009).

There is a large variation in salivary flow and composition between individuals (Heinzerling et al. 2008; Lugaz et al. 2005). Given the importance of saliva on taste perception, these inter-individual differences in salivation rate and composition may also influence how a stimulus is perceived. Norris et al. (1984) grouped subjects participating in a study according to their salivary flow rate and showed that subjects with a high flow rate had a higher taste threshold then subjects with a low flow rate. One possible explanation for this would be that a dilution of the stimulus occurs. We therefore hypothesise that altering the salivary flow rate has an impact on the perceived taste intensity. To test this hypothesis, we controlled the in-vivo saliva release during consumption of various taste solutions by

sealing off the parotid ducts with two Lashley cups. These Lashley cups allowed the collection of secreted saliva preventing the saliva from being released into the mouth. Alternatively, artificial saliva was added back into the mouth of the subjects at well-controlled flow rates, allowing an intra-individual evaluation of taste intensities as a function of salivation rate. This method enabled an individually tuned delivery of artificial saliva at the location where saliva is normally secreted.

In addition, food products containing more than one taste modality are subject to taste-taste interactions (Keast and Breslin 2003; Pangborn 1960). This means that the perceived intensity of a taste attribute related to one tastant is influenced by the presence of another tastant. For instance, the sweetness of a given sucrose solution is generally suppressed by the addition of sour-tasting citric acid (Keast and Breslin 2003). Since different tastant solutions induce different salivation rates, and altering the salivary flow rate might affect the perceived taste intensity we hypothesise that taste-taste interactions are at least in part caused by an altered salivary flow rate. By comparing the perceived intensity of the binary solutions, under different saliva conditions, we could critically test this hypothesis.

4.2 Materials and methods

4.2.1 Stimuli

The stimuli consisted of tastants dissolved in demineralised water (Table 4.1). Four basic tastes (sour, salt, bitter and sweet) were evaluated and two binary taste mixtures (sour / salty and sour / sweet) along with demineralised water as a reference stimulus. The concentration of each stimulus was 10 times stronger than the taste thresholds reported by Amerine et al. (1965).

4.2.2 Subjects

The tastant stimuli were evaluated by seven healthy subjects (6 female and 1 male, aged 51.0±9.1) who did not have any taste disorders and did not use medication that could affect taste, smell or salivary flow. All subjects gave written informed consent prior to the study.

Table 4.1 – Stimuli and concentrations

Taste	Stimuli	Concentration [mM]
Sour	Citric acid	7.9
Salty	NaCl	100
Bitter	$MgSO_4$	46
Sweet	Sucrose	100
Sour + Salty	Citric acid + NaCl	7.9 + 100
Sour + Sweet	Citric acid + Sucrose	7.9 +100

4.2.3 Salivary flow

Parotid saliva was collected using two modified Lashley cups placed over each parotid duct. The Lashley cup (Figure 4.1) is a non-invasive method for collecting parotid saliva (Neyraud et al. 2009). The Lashley cup is fixed to the mucosa on the inside of the cheek by vacuum and the collected saliva flows out through a tube. In this study the Lashley cups prevented the secretion of parotid saliva into the mouth. In addition, it was possible to measure the flow rate of the secreted saliva with a liquid mass flow meter directly connected to the outlet of the Lashley cup (ASL 1430-16, Sensirion, Stafa, Switzerland). An additional tube was fitted to the Lashley cup so that artificial saliva could be delivered into the mouth at the same point as where the saliva would normally flow out.

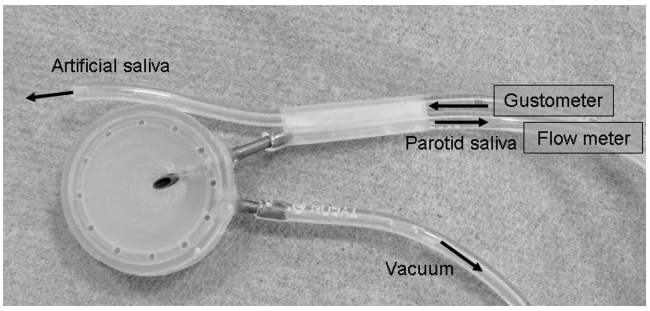

Figure 4.1 – Picture of the modified Lashley cup with the additional tube for delivery of artificial saliva into the mouth. The diameter of the disc is 22 mm

4.2.4 Artificial saliva

A buffered salt solution was used to mimic parotid saliva (Boland et al. 2004). The so-called artificial saliva consisted of: $NaHCO_3$ (5.208 g/L), $K_2HPO_4 * 3H_2O$ (1.369 g/L), NaCl (0.877 g/L), KCl (0.447 g/L) and $CaCl_2 * 2H_2O$ (0.441 g/L). Mucins were not added to the artificial saliva since mucins are not present in the serous parotid saliva. Alpha-amylase was also not added to the artificial saliva since the tastant stimuli used were not expected to be affected by a starch-hydrolysing enzyme.

4.2.5 Measured flow rates and saliva conditions

Based on the individually measured parotid salivary flow rates, different amounts of the artificial saliva were added into the mouths of the different subjects. The delivery of artificial saliva into the mouth of the subjects was controlled with a gustometer (Bult et al. 2007).

4.2.6 Method

The tastant stimuli, 10 mL, were presented to the subjects in cups in random order. The subjects were instructed to take the stimulus into their mouth, hold it for 20 seconds and then spit it out. Thereafter, the perceived intensity of the tastant stimuli (sourness, saltiness, bitterness and sweetness) was scored by all subjects on a 10 cm line scale, anchored ‘not very intense’ at the left end and ‘very intense’ at the right end.

During the whole session two modified Lashley cups were positioned over the two parotid ducts of the subject. For each subject and each stimulus the salivary flow was measured during the 20 seconds that the stimulus was kept in the mouth. From these measurements the individual flow profiles were derived from which the delivery of the artificial saliva was defined. The subjects evaluated the tastant stimuli while artificial saliva was added into their mouth following their individual flow profiles. The artificial saliva was added according to three different saliva flow conditions. Each saliva flow condition was tested twice in separate sessions. In each session all tastant stimuli were tested in duplicates (Figure 4.2).

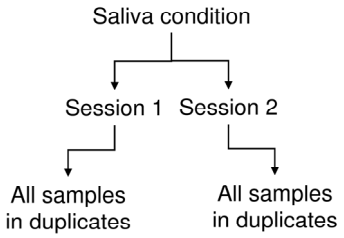

Figure 4.2 – Setup of experimental sessions

4.2.7 Data analysis

The sampling frequency of the salivary flow rate was 1.6 Hz. The perceived intensity scores were normalized within each subject to obtain individual data sets with identical average (M) and standard deviation (SD, Eq. 1)

$$\text{normalized intensity} = \left(\left(\frac{raw\ \text{intensity} - M_{subject}}{SD_{subject}} \right) * SD_{group} \right) + M_{group} \qquad (1)$$

The statistical analysis, ANOVA and post-hoc comparison by Tukey HSD (SPSS, N17, Chicago IL), was performed on the normalised perceived intensity and carried out separately for the four taste qualities (sourness, bitterness, saltiness and sweetness).

The first statistical analysis looked at the effect of the salivary flow conditions on the perceived intensity of each stimulus. The analysis was carried out for citric acid, magnesium sulphate, sodium chloride, sucrose, citric acid + sucrose and citric acid + NaCl independently to determine the effects of salivary flow conditions (fixed factor; main effect), replicate (fixed factor; main effect) and subject (random factor; main effect) on the perceived intensity, thus no interaction effects were analysed. The between subject factors can be seen in table 4.2, N is not the same for all subjects due to missing values.

Table 4.2 – Between subject factors for the first statistical analysis

		N
Condition	No Flow	25
	Normal Flow	24
	Additional Flow	28
Replicate	1	40
	2	37
Subject	A	12
	B	12
	C	10
	D	12
	E	9
	F	10
	G	12

The second statistical analysis looked at the effect of the stimulus composition (taste-taste interactions) and if this effect depends on the salivary flow conditions. The analysis was carried out for citric acid containing stimuli (citric acid, citric acid + sucrose and citric acid + NaCl), sucrose containing stimuli (sucrose and citric acid + sucrose) and sodium chloride containing stimuli (NaCl and citric acid + NaCl). The effects of stimulus (fixed factor; main effect), subject and replicate (random factors; main effects) on perceived intensity were independently evaluated under each condition. Again no interaction effects were analysed. The between subject factors can be seen in table 4.3, N is not the same for all subjects due to missing values.

Table 4.3 – Between subject factors for the second statistical analysis

		N		
		No Flow	Normal Flow	Additional Flow
Sourness				
Stimulus	Water	25	24	28
	Citric acid	25	24	28
	Citric acid + NaCl	25	24	27
	Citric acid + Sucrose	24	24	28
Replicate	1	48	56	55
	2	51	40	56
Subject	A	16	16	16
	B	16	16	16
	C	16	8	16
	D	16	16	16
	E	4	16	15
	F	16	8	16
	G	15	16	16
Saltiness				
Stimulus	Water	25	24	28
	NaCl	25	24	28
	Citric acid + NaCl	25	24	27
Replicate	1	36	42	41
	2	39	30	42
Subject	A	12	12	12
	B	12	12	12
	C	12	6	12
	D	12	12	12
	E	3	12	11
	F	12	6	12
	G	12	12	12
Sweetness				
Stimulus	Water	25	24	28
	Sucrose	25	24	28
	Citric acid + Sucrose	24	24	28
Replicate	1	36	42	42
	2	38	30	42
Subject	A	12	12	12
	B	12	12	12
	C	12	6	12
	D	12	12	12
	E	3	12	12
	F	12	6	12
	G	11	12	12

4.3 Results

4.3.1 Dilution effect of the different salivary conditions

In order to investigate the effect of saliva amounts on the perceived intensity, a methodology was developed which allowed the use of various salivary flow conditions. Three salivary flow conditions were defined. In the first condition no artificial saliva was added. This means that, since there was no parotid saliva entering the mouth and no artificial saliva, this was the 'no flow' condition. In the second flow condition the artificial saliva was added according to the individual flow profiles for each person and stimulus, the 'normal flow'. In the third flow condition an increased amount of artificial saliva was added, the 'increased flow' (Table 4.4).

Table 4.4 – Description of the different saliva flow conditions

Condition	Description
'No flow'	No artificial saliva added
'Normal flow'	Artificial saliva corresponding to the normal flow of each subject added
'Increased flow'	Artificial saliva of which the average flow rate over time equals the maximum flow for the subject. This typically gives flow rates 2 times the normal flow.

This newly developed method makes it possible to modify the salivary flow specifically for each subject and for each specific stimulus. Because the artificial saliva was added into the mouth close to the parotid duct it mimics how real parotid saliva normally enters the mouth. An overview of the individual flow rates and the dilution effects for the two different saliva flow conditions can be seen in table 4.5 (a and b). The dilution of the tastant was defined as the decrease in tastant concentration after addition of artificial saliva, relative to its original concentration, and was calculated as follows (*Eq. 2*):

$$dilution = \left(\frac{\left(concentration_{in\ cup} - concentration_{in\ mouth} \right)}{concentration_{in\ cup}} \right) \times 100 \qquad (2)$$

This means that the dilution of the stimulus is measured and modulated per person in order to take into account as much as possible the individual differences in salivation.

As expected, citric acid stimulated the highest salivary flow rate, almost twice as much as that stimulated by magnesium sulphate, sodium chloride or sucrose (Table 4.5a and b). Surprisingly magnesium sulphate stimulated similar salivary amounts as sodium chloride and sucrose.

The difference in salivary flow between the single tastes and the binary mixtures was due to the presence of citric acid and was not influenced by the presence of another tastant. Citric acid containing samples all stimulated a similar salivary flow rate.

Similar to what has been reported by others we also saw large variations in salivary flow between individuals. For example the measured salivary flow rate for citric acid ranges from 5 µL/sec to 93 µL/sec. The advantage with our method was that it compensated for these individual differences.

4.3.2 Effect of salivary flow conditions on the perceived intensity

The effect of the salivary flow conditions on the perceived intensity of the tastant stimuli can be seen in Figure 4.3. There was a clear decrease in the perceived intensity of citric acid and sodium chloride with an increase of artificial salivary flow. For sucrose there was a non-significant decrease in taste intensity with the presence of saliva (‘normal flow’ and ‘increased flow’) compared to the absence of saliva (‘no flow’). The bitterness of magnesium sulphate was not affected by the salivary flow conditions.

Table 4.5a – Individually measured salivary flow rates and the dilution effect for the two conditions where saliva was added ('normal flow' and 'increased flow').

Subject	Citric acid			$MgSO_4$			NaCl		
	Measured salivary flow (µL/s)	Dilution effect		Measured salivary flow (µL/s)	Dilution effect		Measured salivary flow (µL/s)	Dilution effect	
		Norm (%)	Increased (%)		Norm (%)	Increased (%)		Norm (%)	Increased (%)
A	92.8	16	18	83.8	14	17	81.8	14	16
B	8.5	2	5	7.5	1	4	2.5	0	1
C	48.4	9	27	20.1	4	14	12.0	2	9
D	41.6	8	21	12.8	2	8	19.2	4	11
E	65.3	12	26	34.1	6	16	37.5	7	17
F	83.2	14	35	25.6	5	14	38.4	7	20
G	5.1	1	3	16.3	3	9	8.4	2	5
Mean	56.6	10	22	30.7	6	12	31.9	6	12
SD	34.1	6	12	25.8	4	5	27.2	5	7

Means of replicates for each subject along with overall mean and standard deviation

Table 4.5b – Individually measured salivary flow rates and the dilution effect for the two conditions where saliva was added ('normal flow' and 'increased flow')

Subject	Sucrose			Citric acid + Sucrose			Citric acid + NaCl		
	Measured salivary flow (µL/s)	Dilution effect		Measured salivary flow (µL/s)	Dilution effect		Measured salivary flow (µL/s)	Dilution effect	
		Norm (%)	Increased (%)		Norm (%)	Increased (%)		Norm (%)	Increased (%)
A	91.1	15	18	94.4	16	18	102.9	17	20
B	2.4	0	1	6.1	1	3	11.6	2	6
C	6.1	1	5	45.6	8	26	64.9	11	34
D	15.7	3	9	58.5	10	27	25.9	5	14
E	21.4	4	11	72.2	13	28	35.5	7	16
F	31.4	6	17	117.1	19	43	51.8	9	25
G	22.7	4	12	20.6	4	11	21.3	4	11
Mean	28.0	5	10	65.6	11	25	48.8	9	19
SD	29.8	5	6	39.3	6	13	31.4	5	9

Means of replicates for each subject along with overall mean and standard deviation

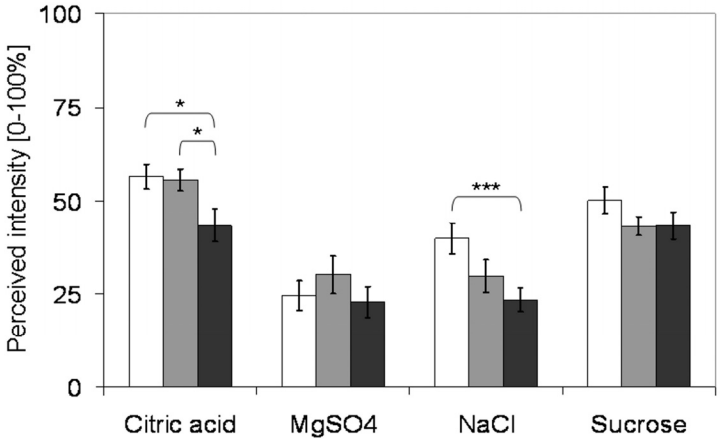

Figure 4.3 - Perceived intensity of citric acid, magnesium sulphate, sodium chloride and sucrose for the three salivary flow conditions; 'no flow' (white bars), 'normal flow' (grey bars) and 'increased flow' (black bars). The bars show the average of all assessors and replicates. Error bars equal the standard error of the mean and * shows the p-values (*p<0.05, **p<0.01, ***p<0.001).

The statistical analysis for citric acid showed a significant effect of salivary flow conditions [$F(2, 67) = 4.560$, $p = 0.014$] but not of subject and replicate. The statistical analysis for sodium chloride showed a significant effect of both condition [$F(2, 67) = 5.930$, $p = 0.004$] and subject [$F(6, 67) = 9.498$, $p < 0.001$] but not for replicate. Neither magnesium sulphate nor sucrose was significantly affected by the salivary flow conditions or the replicate and only magnesium sulphate showed a significant effect of subject [$F(6, 67) = 12.881$, $p < 0.001$].

4.3.3 Influence of saliva on taste-taste interactions

The taste intensity of the binary solutions showed that taste-taste interactions occurred. For instance, the perceived sourness (4a) was significantly higher for citric acid than for citric acid with sucrose or sodium chloride for the 'no flow' and 'normal flow' condition. The same applied for the perceived sweetness which was significantly higher for sucrose than for citric acid with sucrose under all three flow conditions (4b). However, for the perceived

saltiness no suppression could be seen when sodium chloride was tasted in combination with citric acid under the 'no flow' and 'normal flow' condition (4c).

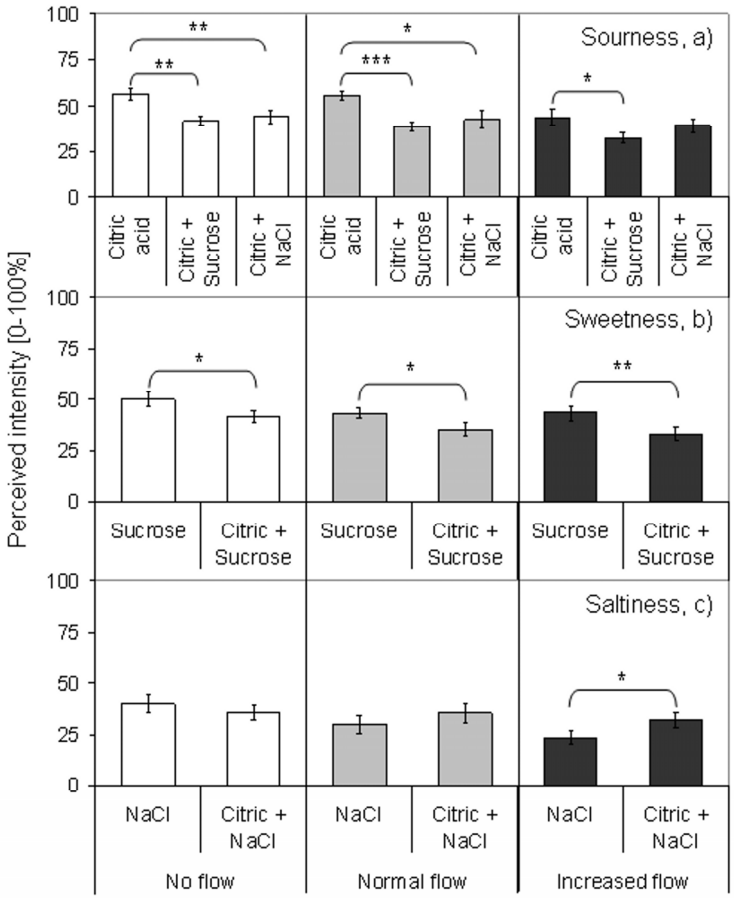

Figure 4.4 - Perceived sourness (a), sweetness (b) and saltiness (c) of basic tastes (citric acid, sucrose and sodium chloride) and binary mixtures of tastants (citric acid + sucrose and citric acid + sodium chloride) for the three saliva conditions; 'no flow' (white bars), 'normal flow' (grey bars) and 'increased flow' (black bars). The bars show the average of all assessors and replicates. Error bars equal the standard error of the mean and * shows the p-values (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

Interestingly the observed taste-taste interactions (4a-b) are not affected by the saliva since they occur both in the 'normal flow' (presence of saliva) and in the 'no flow' condition (absence of saliva). Only the sweetness of sucrose with citric acid was significantly affected by the salivary flow conditions.

Statistical analysis showed that for sourness and sweetness the effects of stimulus was independent of the salivary flow conditions (4a,b) sourness; 'no flow' ($F(3, 88) = 61.742$, $p < 0.001$), 'normal flow' ($F(3, 85) = 48.514$, $p < 0.001$) and 'increased flow' ($F(3, 100) = 45.524$, $p < 0.001$)], [sweetness; 'no flow' ($F(2, 64) = 115.779$, $p < 0.001$), 'normal flow' ($F(2, 62) = 95.008$, $p < 0.001$), and 'increased flow' ($F(2, 74) = 87.741$, $p < 0.001$)]. Saltiness only showed a significant effect for stimulus under the 'increased flow' condition (4c) [$F(2, 73) = 41.124$, $p < 0.001$]. The effect of salivary flow conditions on the different tastant mixtures only showed significant differences between the 'no flow' and 'increased flow' condition for citric acid with sucrose [$p = 0.035$].

4.4 Discussion

Previous studies looking at the role of saliva on taste perception (Norris et al. 1984; Bonnans and Noble 1995; Lugaz et al. 2005) have compared different groups of people (healthy versus ill, old versus young, much saliva versus low amounts of saliva). Although these studies show the link between the amount of saliva and perception, comparisons between people merely suggest a relation between the two. Each person probably adapts to his own salivary flow rate. In a study by Engelen et al. (2003) different amounts of saliva were added to the stimulus. However, the saliva amounts added were the same for each individual. This implies that for an individual with a low flow, extreme amounts were added and for a person with a high flow the added amounts might have gone unnoticed. In our study the artificial saliva was added at individually adjusted flow rates allowing an intra-individual evaluation of taste intensities as a function of salivation rate.

Baek et al. (1999) described how sensory perception of volatiles is related to the rate of change of concentration. We hypothesised that taste perception, in a similar way, is related to temporal contrast. Salivation is continuous and therefore continuously decreases

the concentration of the stimulus in the mouth. It can further be hypothesised that the location of this dilution is also of importance. Studying perception under spatial and temporal contrast is therefore an important difference between this and previous studies. In the study by Engelen et al. (2003) the dilution took place outside the mouth. In their study the stimulus was presented on spoons and two fixed amounts of liquid (water, α-amylase or saliva) were added directly to the spoon prior to digestion. They found none, or only small effects on a number of taste- and mouth feel-attributes.

The results from our study show that the perceived intensity of sourness and saltiness can be modified by a change in the salivary flow. However, the perception of sweetness and bitterness remained unaffected. It is known from studies by Laing et al. (1993) that the difference in concentration between two tastant stimuli has to be at least 13 % to be perceived, with some marginal differences between the tastes (sucrose 14 %, sodium chloride 13 % and citric acid 12 %). In our study the difference in dilution, for citric acid, between the 'no flow' (0 %), 'normal flow' (10 %) and 'increased flow' (22 %) condition was above the detectable level as reported by Laing et al. (1993) and this could explain the significant perceivable difference. For sodium chloride the difference in dilution between the 'no flow' condition (0 %) and the 'increased flow' condition (12 %) was just large enough to be perceived as different. For sucrose however, the difference in dilution was just below the detectable level (5 % 'normal flow' and 10 % 'increased flow') and this could probably explain that no significant effects on the perceived intensity were found.

Although the dilution effect for sodium chloride was the same as for magnesium sulphate, the perception of the latter was not affected by the different saliva flow conditions. First of all it was surprising that magnesium sulphate stimulated as much saliva as sodium chloride. In previous studies (unpublished results) magnesium sulphate hardly stimulated any saliva at all. Secondly, it is possible that the perceivable difference in concentration is higher for magnesium sulphate than for sodium chloride, and that we therefore see an effect of dilution on sodium chloride but not magnesium sulphate.

Other explanations might be found in the composition of the saliva. Saliva is known to influence the perception of acids and salts due to its buffering action and salt content (Behrens and Meyerhof 2006). Furthermore, the different tastes stimulate different

taste receptors. Both citric acid and sodium chloride, which were both significantly affected by the salivary flow conditions, activate ion channel receptors. It is possible that the ion channel receptor is more sensitive to the ion composition of saliva and that this might explain our findings.

Taste-taste interactions were observed in our study but were found to be independent from the salivary flow conditions. If taste-taste interactions depended on an increased amount of saliva (resulting from more taste attributes being present) they would not occur under the 'no flow' condition. However, this was the case for both sourness and sweetness. There was no suppression of saltiness under the 'no flow' condition but also not under the 'normal flow' condition.

The suppression of sourness and sweetness in the presence of more than one taste attribute under the 'normal flow' and 'increased flow' condition can also not be explained by the dilution. The difference in dilution is too low to be perceived. The difference in dilution between the sucrose containing stimuli might only have been noticeable for the 'increased flow' condition (sucrose: 10 % and sucrose + citric acid: 25 %). Thus, the taste-taste interactions are not likely to be due to additional amounts of saliva induced by the combination of two taste attributes. It is more likely to be caused by other factors for example competition at the receptor level or cross-modal interactions. We may conclude from this that taste-taste interactions are not explained by additional saliva dilution of the tastant stimuli.

Our study showed that it is possible to modify the perceived sourness and saltiness by increasing the individual salivary flow rate. Furthermore, taste-taste interactions are not explained by the amount of induced saliva since they also occur when no saliva is present. Putting all the results together, saliva is necessary to transport taste molecules to the taste receptor. In case the saliva volume is strongly diminished due to illness, medication or old age, taste molecules might have difficulties reaching the taste receptors. This reduction in salivary flow can result in a reduced taste perception (Spielman 1990). Norris et al. (1984) on the other hand described how (healthy) subjects with a high flow also had a higher taste threshold, meaning that 'too much' saliva also has a taste reducing effect. It is clear that

there is an optimum amount of saliva in relation to taste perception and that this optimum is individual.

4.5 Conclusions

The manuscript presents a new method to individually modify in-mouth saliva delivery to determine the effect of salivation on taste perception. We hypothesised that an altered salivary flow rate has an impact on the perceived intensity. This is true for the perceived sourness and saltiness but not for the perceived bitterness and sweetness. The second hypothesis, stating that taste-taste interactions are partly caused by an additional amount of saliva, was not confirmed in this study. Taste-taste interactions are likely to be due to other factors.

4.6 Acknowledgements

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Chapter 5

Taste-texture interactions: tastant release as main explanation, rather than impact of salivation or perceptual interactions

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C. I. Heinzerling, M. Stieger, J. H. F. Bult, G. Smit (2013). Taste-texture interactions: tastant release, impact of salivation or perceptual interactions, submitted.

Abstract

Taste-texture interactions have often been described in the literature, but their nature is not yet fully understood. It has been suggested that the taste molecules may not be fully available, either because of decreased tastant release from the texture or because of dilutions or interactions with saliva. Another suggestion is that the reduced taste intensity is caused by cross-modal interactions. In this study, the taste was separated from the texture and the salivary flow was measured continuously as a response to the manipulation of the sample. In this way the tastant availability could be controlled. Results show that there are no texture effects on the perceived taste intensity under these controlled conditions. Therefore, we draw the conclusion that taste-texture interactions can best be explained by tastant release effects rather than by dilution effects or cross-modal.

5.1 Introduction

Sensory perception of food is a highly complex process. During eating, the food product is manipulated orally, leading to structural changes that partly depend on the individual. The perceived sensory properties of the food are affected by chemical or physical interactions within the food matrix (Bonnans and Noble 1995; Christensen et al. 1987; Ferry et al. 2004; Heinzerling et al. 2008) as well as by interaction of the senses on a psychological level (Cook et al. 2002; Delwiche and O'Mahony 1996; Guinard et al. 1997; Lugaz et al. 2005; Matsuo 2000; Norris et al. 1984; Weel et al. 2002). One such interaction, for which no consensus on its origins is reached yet, is the taste-texture interaction. Taste-texture interactions consist typically of a suppression of the perceived taste intensity with an increase in viscosity or hardness of the food matrix (Arabie and Moskowitz 1971; Christensen 1977; Koliandris et al. 2008; Mackey and Valassi 1956; Malone et al. 2003).

There are two different hypotheses concerning the origin of taste-texture interactions. The first one relates to the availability of taste molecules. When a tastant's mobility is limited by the presence of thickeners in a viscous product, its availability at the taste receptor will be less than when present in water, and the perceived taste intensity will be reduced accordingly. Various studies have looked at the effect of different thickeners on the perceived taste intensity. While some in-vitro studies show that there is a difference in tastant release (Bayarri et al. 2001; Boland et al. 2004; Brossard et al. 2006; Koliandris et al. 2008; Malone et al. 2003; Sala et al. 2010; Tournier et al. 2009), only a few studies have looked at the effect in-vivo (Davidson et al. 1999; Neyraud et al. 2003). In-vivo measurements of tastant release are not simple and no standard operating procedure does yet exist. For the in-vivo assessment of sodium release, for example, intra-oral conductivity measurements have been conducted (Neyraud et al. 2003). This technique, however, exhibits limitations in accuracy as saliva contains other salts as well which have to be taken into account for these types of measurements (Delwiche and O'Mahony 1996; Matsuo 2000; Spielman 1990). A few attempts have been made to sample whole mouth saliva after ingestion for in-vitro determination of the tastant concentration in the whole mouth saliva mixture (Davidson et al. 1999). However, the flow, and therefore also the composition, of whole mouth saliva largely depends on the sampling technique and no validated method is

available to date. Furthermore, the tastant chemistry is influenced by the volume and composition of the saliva released during ingestion. Compositional effects include an alteration of the stimulus chemistry through the buffering capacity and neutral pH of saliva as well as through enzymatic breakdown of starch and fat (Christensen et al. 1987; Heinzerling et al. 2008; Norris et al. 1984). The mixing with saliva and the changes in stimulus chemistry also influences how the stimulus is perceived (Bonnans and Noble 1995; Christensen et al. 1987; Delwiche and O'Mahony 1996; Guinard et al. 1997; Heinzerling et al. 2008; Lugaz et al. 2005; Matsuo 2000; Norris et al. 1984). As we demonstrated previously, the perceived taste intensity can be reduced by an individual increase in saliva amounts (Heinzerling et al. 2011). Salivation is additionally stimulated by tongue movement and chewing (Anderson and Hector 1987). It is therefore likely that salivation also plays a role in the occurrence of taste-texture interactions and on the availability and chemistry of the taste molecules (Christensen et al. 1987; Delwiche and O'Mahony 1996; Guinard et al. 1997; Heinzerling et al. 2011; Matsuo 2000; Norris et al. 1984; Spielman 1990).

The second hypothesis concerns cognitive interactions. Cross-modal perceptual interactions occur when impressions from different sensory modalities, like for instance flavour and texture, affect each other while being processed at the same time (Bult et al. 2007; Cook et al. 2002; Juteau et al. 2004; Weel et al. 2002).

Cross-modal perceptual interactions have been suggested as the cause of aroma-texture interactions since several studies have shown that the perception of aroma is independent of the actual aroma release (Bult et al. 2007; Davidson et al. 1999; Visschers et al. 2006). Although comparable experiments have not yet been performed on taste-texture interactions, it could be assumed that taste is affected in a similar way by texture.

In order to unravel the mechanisms that determine taste-texture interactions, it is desirable to control both the release of tastants from the matrix as well as their mixing with saliva during mastication. We therefore compared the perceived taste intensities of two tastant solutions in the presence and absence of two texture stimuli. Inert, tasteless materials were presented as texture stimuli to exclude physical and chemical interactions between the texture stimuli and the taste stimulus. In addition, the parotid saliva flow was measured

continuously during the oral processing of these stimuli, allowing us to relate perceived taste intensity to saliva production and stimulus texture.

5.2 Materials and method

5.2.1 Textures

The two texture stimuli consisted of 5x7x14 mm pieces of unflavoured chewing gum base (Cargill R&D Centre Europe, Vilvoorde, Belgium) and 14x14x14 mm pieces of ethylene-vinyl acetate foam (EVA foam), which is a closed-cell foam used in camping mats and children toys. The mechanical properties of the texture stimuli were determined using uni-axial compression tests (Instron universal testing machine, model 5543, Instron International Ldt., Edegem, Belgium), as described in Sala et al. 2009. The chewing gum base was about 200 times stiffer (34777 kPa) than the EVA foam (165 kPa). Furthermore, the chewing gum base was about 4 times less elastic (20.9 %) than the EVA foam (84.6 %). Both materials were inert; they did not break down during chewing and did not absorb any liquid.

5.2.2 Tastant solutions

Sodium chloride and sucrose were dissolved in demineralised water to a concentration of 100 mM. Demineralised water was used as a reference. Stimuli were prepared by combining 2 ml of the tastant solutions (NaCl, sucrose, water) with the textures (EVA foam, chewing gum base, no texture) in a full factor fashion.

5.2.3 Subjects

Eight healthy subjects (5 females and 3 males, aged 47.1±9.0) participated in this study. They did not have any taste disorders and did not use any medication that could affect their saliva production or taste perception. Subjects were paid for their participation and gave written informed consent prior to the study.

5.2.4 Method

The tastant solutions and the textures were presented to the subjects in plastic cups at 2 minute inter-stimulus intervals (Figure 5.1). When the textures were tested together with a tastant the subjects were instructed to first put the texture in their mouth and thereafter the liquid. Orders of stimulus presentation were individually randomised. After completion of a training session to familiarize with the procedure, subjects completed two experimental sessions on subsequent days. During a session all the samples were presented in duplicates.

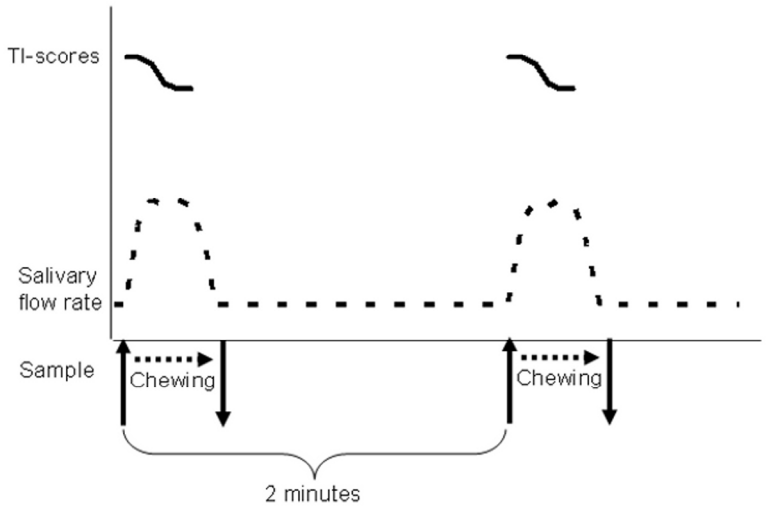

Figure 5.1 - Schematic figure of the set-up of the study

During an experimental session, subjects received timed instructions on a computer screen, indicating when to put a stimulus in their mouth, when to start chewing and when to spit out the stimulus. While chewing, subjects scored the intensity of the stimuli over time (time-intensity) by moving the control of a vertical rating-bar on the computer screen in front of them. The maximum score (100) represented a 'very intense' taste and the minimum score (0) 'no taste'. The subjects were instructed to chew the textures as they would normally chew a chewing gum. During the complete session the

saliva produced by one of the parotid glands was collected with a Lashley cup (Heinzerling et al. 2011) and its flow rate was measured with an ASL 1600-20 liquid mass flow meter (Sensirion, Stafa, Switzerland). After the flow rate was measured the saliva was discarded.

5.2.5 Dilution effect

The dilution of the tastant was defined as the decrease in tastant concentration after addition of saliva, relative to its original concentration, and was calculated as follows (Eq. 1):

$$dilution = \left(\frac{\left(concentration_{\,in\ cup} - concentration_{\,in\ mouth} \right)}{concentration_{\,in\ cup}} \right) \times 100 \qquad (1)$$

The dilution of the stimulus was measured per person in order to take into account as much as possible of the individual differences in salivation.

5.2.6 Data analysis

Each sample was presented in quadruplicates and the intensity scores were recorded for 20 seconds. The sampling frequency of the time intensity software was set to 2 Hz. The sampling rate of the flow meter was 50 Hz. Before data analysis, this sampling rate was compressed to 2 Hz and only flow rate measures from the 20 second time intervals during which stimuli were evaluated were kept for further analysis. In this way, the measured salivary flow rates corresponded to the recorded intensity scores in time. Before statistical testing, intensity ratings and salivary flow rates were normalized within each subject to obtain individually normalized intensity and flow rate scores at identical average (M_{group}) and standard deviation (SD_{group}) (Eq. 2):

$$normalized\ scores = \left(\left(\frac{raw\ score - M_{subject}}{SD_{subject}} \right) * SD_{group} \right) + M_{group} \quad (2)$$

After normalization, the areas under the salivation flow curve (AUCS) and the taste intensity curve (AUCI) were calculated and the maximum salivation rate (MAXS) and the maximum taste intensity score (MAXI) were assessed for further statistical analysis.

Table 5.1 – Between subject factors for the statistical analysis

		N	
		AUC_S/MAX_S	AUC_I/MAX_I
Texture	None	95	96
	EVA foam	93	95
	Chewing gum	95	95
Taste	None	94	96
	NaCl	93	94
	Sucrose	96	96
Replicates	1	72	72
	2	70	72
	3	69	70
	4	72	72
Subjects	A	36	36
	B	35	35
	C	36	36
	D	35	36
	E	34	36
	F	36	36
	G	35	35
	H	36	36

Effects of tastant (3 levels, fixed factor), texture (3 levels, fixed factor), replica (4 replicates, fixed factor) and subjects (8 subjects, random factor) on AUCS, MAXS, AUCI and MAXI were tested for statistical significance by full factorial univariate ANOVA. The between subject factors can be seen in table 5.1, *N* is not the same for all subjects due to missing values.

Post-hoc comparisons between Stimulus categories were performed with Tukey HSD correction for multiple comparisons in a second ANOVA where effects of stimulus (9 levels, fixed factor), replica (4 levels, fixed factor) and subjects (8 levels, random factor) on AUCS, MAXS, AUCI and MAXI were tested. All tests were performed at $\alpha = 0.05$ with SPSS version 17.1 (SPSS Inc, Chicago IL).

5.3 Results

5.3.1 Effects of time

The changes in taste intensity and salivary flow rate with time are shown in figure 5.2. Perceived intensities increased sharply at the beginning, to reach a maximum after about 5 seconds and then remained constant for the remaining 15 seconds (water) or decreased slowly (sodium chloride and sucrose). No taste adaptation was observed in the course of the experiment. After taste stimulation, the salivary flow increased gradually with time. The stimuli containing chewing gum or EVA foam produced steeper inclines of salivation rates over time than the tastants alone. In addition, their salivation rates were fluctuating due to the chewing. The samples without additional textures stimulated less saliva than those with a texture. Overall sodium chloride seemed to stimulate more saliva than sucrose and water.

5.3.2 Saliva – area under the curve and maximum salivary flow rate

Figure 5.3 shows the differences in salivary flow rates, in terms of the area under the curve and the maximum value for the different stimuli. Contributions of different tastants to salivary flow rates followed a similar order as in previous studies: water < sucrose < sodium chloride (Hodson and Linden 2006). There was a clear increase in salivation when a texture was presented together with the tastant solutions. The chewing gum seemed to

stimulate more saliva in total (AUC) than the Ethylene-vinyl acetate, independent on which taste solution it was tested with.

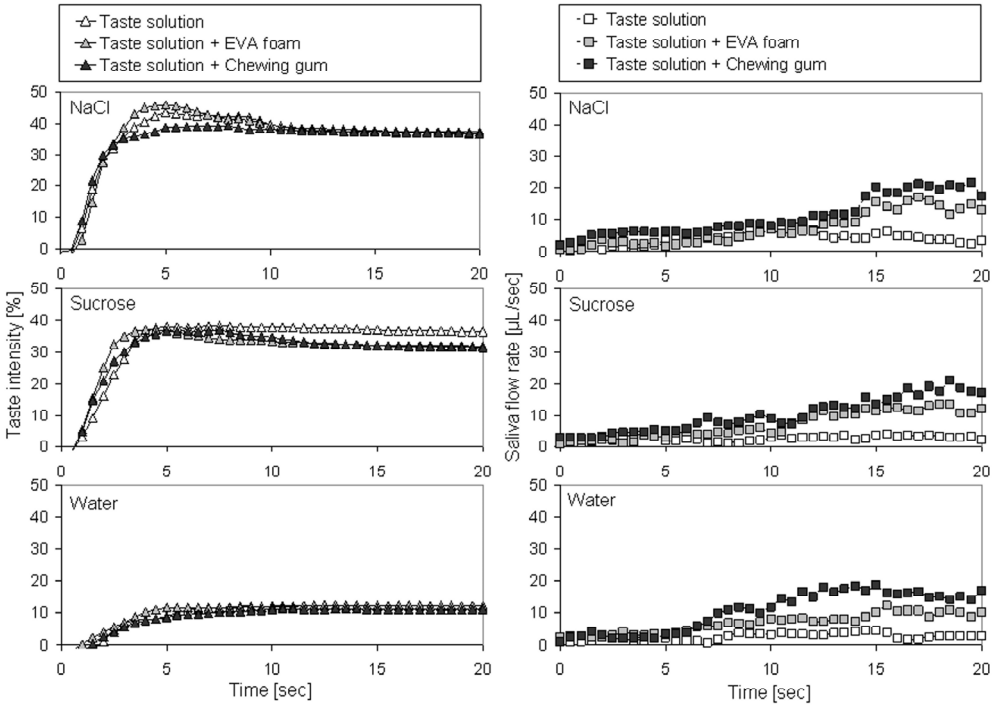

Figure 5.2 – Perceived intensity (triangles) and salivary flow rate (squares) of taste solutions (open symbols), taste solutions with Ethylene-vinyl acetate (semi-closed symbols) and taste solutions with chewing gum (closed symbols) as a function of time. Data points are means of all assessors and replicates.

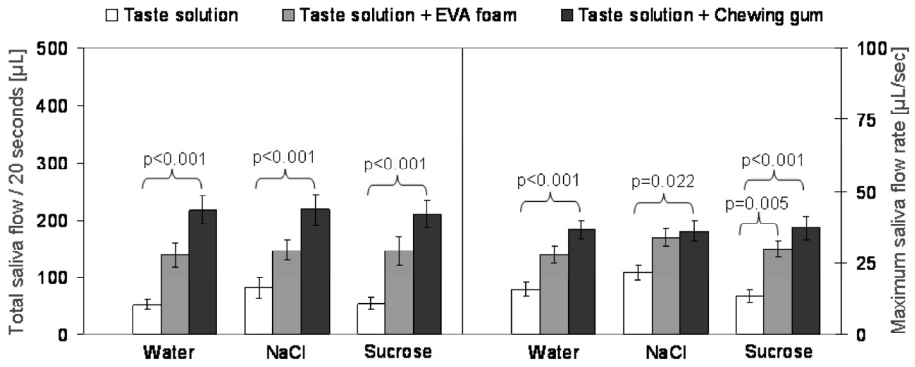

Figure 5.3 - Total saliva flow (left) and maximum saliva flow rate (right) induced by stimulation with different textures and taste solutions. Means of all assessors and replicates, error bars equal the standard error of the mean.

The statistical analysis showed that the total saliva amount (AUCS) was significantly affected by texture [$F(2, 264) = 43.554$, $p < 0.001$] but not by tastant. Furthermore, it was significantly affected by replicates [$F(3, 264) = 3.345$, $p = 0.020$] but not by subject. No 2-way or 3-way interactions were observed. The maximum salivary flow (MAXS) was also significantly affected by texture [$F(2, 264) = 36.662$, $p < 0.001$] and replicates [$F(3, 264) = 6.784$, $p < 0.001$], but not by tastant and subject. Again there were no interaction effects. Post-hoc comparison showed a significant increase in salivation rate, for both total flow (AUC) and maximum flow (MAX), when the taste was tested in combination with the chewing gum (water, sodium chloride and sucrose). Furthermore, the maximum flow for sucrose in combination with the Ethylene-vinyl acetate was significantly higher than the maximum flow for sucrose without additional texture. No further differences in flow between the solutions and the solutions with a texture were observed.

The stimulated saliva caused a dilution of the taste solution in the mouth. The various dilution ratios averaged per stimulus are shown in table 5.2. The highest dilution was caused by the addition of the chewing gum. However, on average the difference between the solutions and the two textures was not very large. The taste solution on its own

caused a dilution effect of 3 %, whereas the two textures caused a dilution effect of 6 % (EVA foam) respectively 9 % (chewing gum).

Table 5.2 – Dilution effect (%) with saliva for the different stimuli (means of all assessors and replicates)

		Taste		
		None (water)	NaCl	Sucrose
Texture	None	2.5 (SEM 0.4)	3.7 (SEM 0.8)	2.6 (SEM 0.5)
	EVA foam	6.2 (SEM 0.9)	6.6 (SEM 0.8)	6.4 (SEM 1.0)
	Chewing gum	9.5 (SEM 0.9)	9.7 (SEM 1.0)	9.2 (SEM 0.9)

5.3.3 Taste – area under the curve and maximum intensity

Taste intensities for the different stimuli, expressed as the area under the curve and the maximum intensity, are shown in figure 5.4. There was no clear effect of the addition of a texture on the perceived intensity.

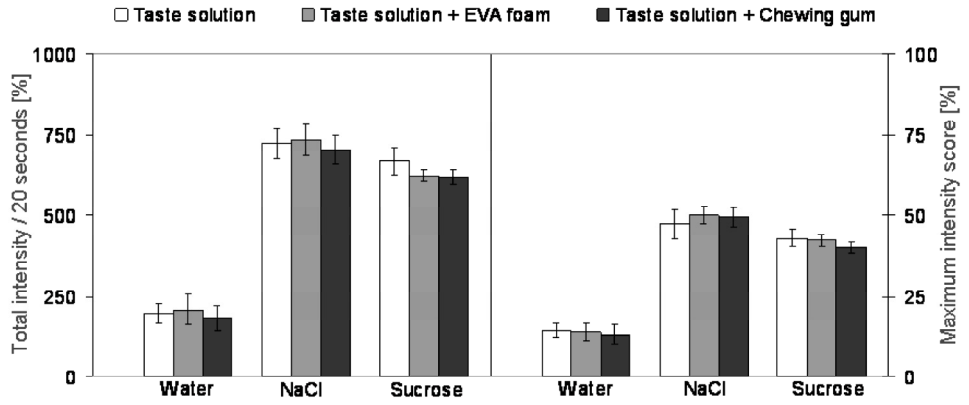

Figure 5.4 - Taste intensity scores for the taste solutions without and with the two textures. Means of all assessors and replicates, error bars equal the standard error of the mean.

Statistical analyses showed that the total intensity (AUCI) was affected by tastant [$F(2, 267) = 135.476$, $p < 0.001$] but not by texture. It was also significantly affected by replicates [$F(3, 267) = 3.181$, $p = 0.024$] but not by subject. There were also no interaction effects of texture x tastant on total intensity. The maximum intensity (MAXI) was significantly affected by tastant [$F(2, 267) = 132.514$, $p < 0.001$] and subject [$F(7, 267) = 5.185$, $p < 0.001$] but not by texture or replicate. Again no interaction effects were observed. Post-hoc comparison showed that the significant stimulus effect was seen only between the different taste solutions (water, sodium chloride and sucrose) and not between the samples with or without an accompanying texture. In spite of the fact that saliva production was affected by tastant, there was no significant effect of the texture on the perceived taste intensity.

5.4 Discussion

Studies showed that texture affects the release of tastants from the matrix and that this also has an effect on the taste perception (Bayarri et al. 2007; Boland et al. 2004; Sala et al. 2010). Most studies have looked at in-vitro tastant release (Bayarri et al. 2001; Brossard et al. 2006; Koliandris et al. 2008; Malone et al. 2003; Sala et al. 2010; Tournier et al. 2009). Sala et al. 2010 for example, showed that gels with a serum release of 12 % were perceived equally sweet as gels with 30 % more sugar and only 2 % serum release. Bayarri et al. 2007 tested different oil/water emulsions with the same viscosity but different oil content. Increasing oil content affected the tastant release and had a significant decreasing effect on the perceived sweetness. In-vivo tastant release studies, both using chewing gums, also showed a correlation between the measured release of the tastant and the perceived intensity (Davidson et al. 1999; Neyraud et al. 2003). In our study taste-texture interactions could be studied independently of tastant release, since the texture stimuli consisted of chemically inert materials. This could also be the explanation to why no taste-texture interactions were observed. However, as seen in our previous study (Heinzerling et al. 2011) the taste perception is also affected by the tastant composition in the mouth and thus by the saliva. The previous study (Heinzerling et al. 2011) showed that there was a significant decrease in perceived sourness and saltiness with an increase in dilution. In this

case the tastant was diluted by the addition of artificial saliva into the mouth at individual flow rates while at the same time the parotid saliva was prevented from entering the mouth. The averaged dilution effect was 6 and 14 % and there was also a situation were no artificial saliva was added, a 0 % dilution. The difference between the 0 % and 14 % dilutions was big enough to be significantly perceived. In the study presented here the dilution effect was 3 % for the pure solution, 6 % for the taste solution combined with the EVA-foam and 9 % for the taste solution and the chewing gum. The dilution effect might therefore be too low to cause a significant reduction in taste intensity. On the other hand saliva from only one parotid gland caused this dilution effect. In a normal eating situation the parotid saliva would not have been collected from one of the glands. In that case, the amounts of saliva actually entering the mouth would have been twice of what was now measured (equalling 6 % for the pure solution, 12 % for the taste solution + EVA-foam and 18 % for the taste solution + chewing gum). Therefore a dilution effect cannot be completely ruled out at least between the pure taste solution and the taste solution with chewing gum. Still, this high dilution effect would only be caused by a hard product and most other studies observed effects with viscous materials, which most likely did not stimulate these high amounts of saliva.

Another explanation for taste-texture interactions are cross-modal perceptual interactions, which, if they exist, we would expect to be detected in this study set-up. Aroma perception is generally thought to be caused by cross-modal interactions. Studies show that the perceived aroma has little in common with the actual aroma release (Bult et al. 2007; Cook et al. 2002; Juteau et al. 2004; Weel et al. 2002). Weel et al. 2002 only studied aroma-texture interactions by using (tasteless) gels with different textural properties. They showed that the perceived aroma intensity changed significantly with gel stiffness, despite the fact that volatile release remained unaffected. Other studies separated between the texture and the aroma by using a tasteless gel and adding the aroma ortho- or retronasally (Bult et al. 2007; Visschers et al. 2006). The viscosity still suppresses aroma perception even when in-nose aroma concentrations are kept constant. For aroma-texture stimuli, it is often observed that the aroma, although processed in the nose, perceptually emanates from the mouth (Small et al. 2005). This blending of taste-texture impressions

with olfaction may explain why perceptual aroma-texture and aroma-taste interactions are observed, even though the (retronasal) aroma does not emanate from the actual oral stimulus (Bult et al. 2007). In the setting by Bult et al. 2007 and Visschers et al. 2006, despite being separated, the perceived aroma intensity was still affected by the texture and not by the actual aroma delivery. Contrary to the apparent blending of oral stimulus impressions with the concurrent aroma, it is not yet known too which extent oral texture and oral taste blend perceptually. It is possible that the separation of taste and texture as we achieved it in this study disrupts the perceptual blending of these modalities to the extent that perceptual interactions are precluded. This would then explain why we did not observe any cross-modal taste-texture interactions with the used model stimuli. However, there are also indications that aroma is more sensitive to the presence of other sensory stimuli than taste. Aroma can for example be perceived although it is not present and it is known to be influenced by the presence of taste. Davidson et al. 1999 showed that the perceived menthol in chewing gum actually followed the release curve of sugar and not of menthol. Congruent taste-aroma combinations can enhance the perceived intensity, just as incongruent combinations can decrease the intensity (Bayarri et al. 2007; Lawrence et al. 2009; Pfeiffer et al. 2005; Tournier et al. 2009). Several studies (Labbe et al. 2007; Schifferstein and Verlegh 1996) have shown that it is possible to increase the sweetness or saltiness of a product by the addition of aroma, but only if sugar and salt is present. Taste seems to be more dominant than aroma and it could be that taste is also more dominant than texture, when it comes to cross-modal interactions. In that case, although cross-modal interactions occur for aroma-texture interactions they do not occur for taste-texture interactions.

Summarising the results, in our set-up no taste-texture interactions were found. Reduced tastant release was not a factor in our study as the taste was clearly separated from the texture. The impact of salivation is questionable as the dilution effect was probably too low to be noticeable. In terms of perceptual interactions, no cross-modal interactions occurred, either because there was no perceptual blending of taste and texture in our set-up, or because taste, unlike aroma, is not affected by the perceived texture.

5.5 Acknowledgements

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Chapter 6

General discussion

6.1 Summary of main findings

This thesis has focused at the different interactions between saliva, taste and texture (Figure 6.1). Interactions are always two sided. In this case, the taste and texture stimulates saliva with a certain flow rate and composition, but the saliva flow rate and composition will also affect how the stimulating taste and texture is perceived. Some of these interactional effects were measured and methods for manipulating the salivary flow and composition causing these effects were developed. One of the aims was to control the individual variation without completely excluding individual differences.

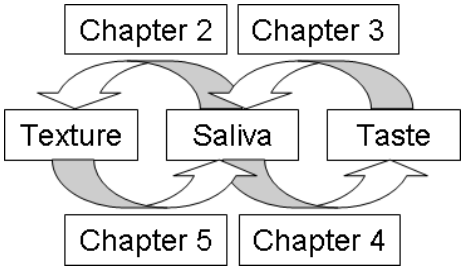

Figure 6.1 – Structure of the thesis; showing the various interactions and their corresponding chapters

In chapter 2 it was shown that reduced α-amylase activity increased the perceived thickness; however, individual differences (not being measured) explained most of the variation in perception. Acidification with citric acid resulted in saliva that was more concentrated in α-amylase. The greatest protein secretion during salivation was induced by citric acid. The protein amount was dependant on the tastant, but independent of the salivary flow rate. The pH of saliva was only depending on the salivary flow rate and not on the type of stimulus (Chapter 3). There was a significant decrease in perceived sourness and saltiness intensity for increasing salivation but not for perceived sweetness and bitterness. This cannot be solely a dilution effect, since sweetness was not affected at a similar salivation rate. The composition of saliva and the type of taste receptor (ion channel vs. G-protein coupled receptors) can be considered as an explanation. Suppression of sweetness and saltiness in binary mixtures were not affected by increased salivation

(Chapter 4). Taste-texture interactions are presumably defined by the extent of tastant release and not by cross-modal interactions or dilution with saliva. This because the perceived intensity did not change with the addition of an inert texture, even though there was a clear increase in salivation (Chapter 5).

6.2 Scientific impact

The overall aim of this thesis was to show what interactions are present and what impact saliva has on these interactions. The results from the work presented in this thesis, together with what is known from literature has been compiled in table 6.1. Some effects (texture-texture and aroma-aroma) have not been observed in this thesis work and they have so far not been described in the literature either.

Starting at the top, reading from left to right, first described are the effects of saliva. Saliva flow rate defines the salivary pH (Chapter 3). Saliva flow and composition also affects the taste. Christensen et al. 1987 described that a decreased flow rate (e.g. less than normal due to illness/old age/medication) results in a decreased taste perception. Our study (Chapter 4) supported these findings and also showed that an increased flow rate (e.g. more than normal) has a decreasing effect on taste perception. This suggests that there is an optimum salivary flow rate in respect of taste perception. Furthermore, the composition of saliva is also important for the taste perception. Saliva has a neutral pH and consists of bicarbonate ions and sodium salts (Christensen et al. 1987; Larsen et al. 1999; Wakim et al. 1969). This influences both the sour perception through buffering, and the salt perception through determining the salt taste threshold (Christensen et al. 1987; Lugaz et al. 2005). Saliva flow and composition also affects the texture of foods – both the perceived thickness and the actual thickness (Chapter 2). Aroma-interactions have not been investigated in this thesis, however it also plays an important role in food perception and aroma-interactions are interesting to compare to taste-interactions. Regarding the role of saliva on aroma perception, no effect has been shown for the sensory perception or for the actual aroma release.

Continuing at the second row, reading from left to right, we have the effects of taste on salivary flow and composition. Different tastes induce different amounts of saliva,

and different salivary flow rates induce different pH (Hodson and Linden 2006; Speirs 1971, Chapter 3). The protein concentration, however, is independent on flow rate and instead dependant on tastant type (Chapter 3). Taste-taste interactions do occur and are probably not influenced by the salivation. They are more likely caused by cross-modal interactions (Chapter 4). No effects of taste on texture were observed in our work and no reference to this has been found in the literature. Taste does effect aroma perception. Beyond that it can sometimes be mistaken for aroma, as described in the study by (Davidson et al. 1999). In that case a decrease in tastant concentration was mistaken for a decrease in aroma concentration although the aroma release remained constant or was added separately.

The third row shows the effect of texture on saliva. Chewing is known to stimulate salivation, especially from the parotid gland as it is located so that jaw clenching mechanically affects the gland itself (Anderson and Hector 1987, chapter 5). With increasing salivary flow the pH of the saliva is increasing as well. The salivary flow rate is positively correlated with the pH of the saliva (Chapter 2). Texture-taste interactions show a decrease in taste perception with an increasing viscosity which can be explained by a reduced tastant availability (Arabie and Moskowitz 1971; Bayarri et al. 2001; Boland et al. 2004; Brossard et al. 2006; Christensen 1977; Koliandris et al. 2008; Mackey and Valassi 1956; Malone et al. 2003; Sala et al. 2010; Tournier et al. 2009). Texture-aroma interactions are on the other hand caused by cross-modal interactions and independent of aroma availability (Bult et al. 2007; Cook et al. 2002; Juteau et al. 2004; Visschers et al. 2006; Weel et al. 2002).

Does aroma affect the salivary flow rate (last row)? Aroma does stimulate submandibular saliva but not parotid saliva (Lee and Linden 1991; Lee and Linden 1992). Aroma does effect taste perception (Labbe et al. 2007; Schifferstein and Verlegh 1996). It can have an enhancing effect if taste and aroma are congruent (Bayarri et al. 2007; Lawrence et al. 2009; Pfeiffer et al. 2005; Tournier et al. 2009). However, this enhancing effect can only occur if the taste is present, aroma cannot solely substitute taste. Aroma can also affect the texture perception as shown for example in a study by Bult et al. 2007 showing that a buttery aroma affected the perceived creaminess.

Table 6.1 – Overview of interactions extracted from this thesis and information known from the literature (in *italic*)

	Saliva	Taste	Texture	Aroma
Saliva	Saliva flow rate defines the salivary pH [1]	*Decreased amounts of saliva = decreased taste perception* [2] Increased amounts of saliva = decreased taste perception [3]	Amylase activity affects both the perceived thickness and the actual thickness of the food [4]	*Not known to affect the aroma release*
Taste	Different tastes induce different salivation rates and protein concentration is dependent on tastant type, independent of flow rate [1]	Taste-taste interactions - no influence of saliva [3]	?	*Taste can be mistaken for aroma – a decrease in taste concentration can be mistaken for a decrease in aroma concentration* [5]
Texture	Chewing stimulates salivation [6]	Decrease in taste perception with increasing viscosity is explained by reduced tastant availability [6]	N/A	*Aroma-texture interactions = cross-modal interactions* [7]
Aroma	*Not known* [8]	*Taste enhancing if congruent* [9] *Aroma cannot solely substitute taste*	*Aroma can increase the perceived texture* [10]	N/A

References to table: [1] Chapter 3 of this thesis, [2] Christiansen et al. 1987, [3] Chapter 4 of this thesis, [4] Chapter 2 of this thesis, [5] Davidson et al. 1999, [6] Chapter 5 of this thesis, [7] Visschers et al. 2006, [8] Lee and Linden 1991; 1992, [9] Bayarri et al. 2007, [10] Bult et al. 2007

6.3 Impact on other topics

Saliva is important for many things, not just food ingestion. It is important for our mouth and teeth, that the oral biology is maintained (Van Nieuw Amerongen et al. 2004). It is important for the ingestion and the health maintenance of our stomach, patients in coma experience problems due to the fact that they cannot swallow their saliva (Björne 2005). Humans swallow about 0.5-1.5 litres of saliva every day (Van Nieuw Amerongen et al. 2004). The studies in this thesis have shown to be important in other fields outside sensory perception as such.

6.3.1 Teeth maintenance

Alpha amylase is an enzyme which breaks down starch. It is active at a pH of 7. When the food has been swallowed and enters the stomach the α-amylase will be inactivated by the stomach acid (Evans et al. 1986; Merritt and Karn 1977; Wakim et al. 1969). The starch break down of the food for metabolic reasons is therefore limited to the time the food matrix is present in the mouth and throat. However, food rests also gets stuck in our teeth. If the starch is not broken down it will become a good nutrient medium for bacteria, which in turn will produce acids as a bi-product. These acids can break down the dental enamel and cause caries (Van Nieuw Amerongen et al. 2004). The amount of α-amylase in saliva is highly individual and the rate of caries is also highly individual (Kivela et al. 1997, Englander et al. 1958). That saliva amounts and composition, especially pH and buffering capacity, are of importance for the caries status of a person is known (Englander et al. 1958; Van Nieuw Amerongen et al. 2004). But perhaps salivary enzymes should also be seen as an important factor. In this case the brushing of teeth after a starch rich meal or even a low starch food diets might be advisable for low α-amylase producing individuals.

6.3.2 Xerostomia

Dry mouth, hyposalivation or xerostomia, which is the medical term for the symptom, can be caused by a variety of factors such as age, illness, and medication (Van Nieuw

Amerongen et al. 2004). The dry mouth is mainly a problem for the oral health as the lack of saliva will cause demineralization of the teeth and infection of the mucosa (Van Nieuw Amerongen et al. 2004). However, the lack of saliva also has a diminishing impact on the taste (Norris et al. 1984; Spielman 1990). As already mentioned, saliva is necessary for taste perception as it transports the taste molecules to the taste receptors (Matsuo 2000; Van Nieuw Amerongen et al. 2004). If no saliva or not sufficient amounts of saliva is present this will prevent this transport resulting in a diminished taste perception (Norris et al. 1984; Spielman 1990). Xerostomia is generally treated with saliva inducing substances such as sour tasting lozenges or chewing gum (Van Nieuw Amerongen et al. 2004). The general aim is to increase the individual's saliva production. If this is not possible artificial saliva can also be used (Van Nieuw Amerongen et al. 2004). However, our study (Chapter 4) also shows that too much saliva decreases the taste perception, at least for sourness and saltiness. It is therefore important to find an optimum saliva amount in relation to taste perception when treating xerostomia.

6.3.3 Dysphagia

Dysphagia is the medical term describing the difficulty to swallow (Brady 2008). It is a complex condition caused for example by obstructions like tumours, diseases of the muscles in the throat, brain diseases or stroke, and by xerostomia (Brady 2008). The treatment is highly dependent on the cause and ranges from surgical removal or medication to treatment with artificial saliva (Brady 2008). When dysphagia is caused by a stroke, it is often treated by thickening the liquid foods, as thin liquids are more likely to be aspirated (Brady 2008). Sasaki and Leder (2009) published a comment on our study (Chapter 2) in the journal Dysphagia. Since the saliva changes the viscosity of starch-containing foods it will also have an impact on the products used in the diagnosis and treatment of dysphagia. This comment shows how closely related the various topics are.

6.4 Suggestions for future research

An argument that was often mentioned in discussions about this work was that it is too 'un-natural' and thus might not reflect the real eating situation. The same question can in that case be applied to almost all sensory studies where parameters are controlled or measured. How natural is it to get liquids pumped into your mouth through a tube? How natural is it to score aroma intensity at the same time as you have a tube 10 cm into your nose? How natural is it to sip-and-spit and score the perceived intensity of a food product, sitting in a small cubical in a room full of people? Some things are indeed far away from 'normal' eating behaviour, but the definition of 'natural' is just as individual as many of the other parameters we try to control and measure. What is normal for one person can be highly abnormal for someone else; it is highly influenced by cultural heritage and memory. To chew on a tasteless texture or to have a Lashley cup positioned on the inside of the cheek is not more abnormal or un-natural than what has been done in other sensory studies. However, our methods should continuously be challenged and it is important to question if our results can be interpreted as to reflect a 'normal' situation.

Cross-modal interactions, since they are difficult to actually test, run the risk of becoming an explanation for observations that cannot otherwise be explained. Language is sometimes a limiting factor. Scientists try to use words which are exactly defined. However, what is self-explanatory to a scientist might not be so for a consumer. Consumers might not be able to differentiate between for example taste and flavour. If only flavour is asked and the consumer associates this with taste *and* aroma while the scientist only associates it with aroma then the wrong conclusions will be drawn.

Most of the variation in perception is explained by individual differences (58 % from Chapter 2). In this study the effect of saliva (dilution, pH, α-amylase activity and bolus temperature) was separated from the individual differences and separated it accounted for 11 % of the variation. This seems to be a small effect. On the other hand, what other effects are included in the individual differences of 58 %? The time the food is processed in the mouth, mouth movements, taste bud morphology, cultural heritage and memory? If these other effects could also be defined and measured, how much of the variation in perception would they explain? It could be that saliva is the comparatively largest part.

Would it be possible to define and measure these other parameters? Yes, everything is possible if the right method can be found. How do you get a texture without a taste? How can you separate the taste from the texture? How do you control the amounts of saliva in the mouth? By using a non-food item, a 'texture', and adding the taste on the side and by blocking the parotid ducts with Lashley cups. Try to think 'outside the box' when trying to find solutions for how parameters can be tested. "The level of success is limited by our own imagination" (Aesop, 620 BC-560 BC).

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Summary

The perception of food is influenced by various parameters and subject to large inter-individual differences. What we perceive is not the same because each individual is different. Each individual has a different taste-bud and smell-receptor physiology, different cultural heritage and different memories. The parameter saliva provides an additional source of variation, since it will change the chemical and structural composition of the food mixture during oral manipulation. Saliva volume and composition vary widely among people and also vary during eating. Therefore, the dilution and mixing of food with saliva determines the extent of food-saliva interactions and thus also how the food item is perceived.

To what extent each of these parameters explain the intra-individual variation observed is not clear. The aim of this thesis is to investigate one of those factors in more detail – the role of saliva. Saliva flow and composition can be measured for each individual and it can be manipulated in a controlled fashion. It is known from the literature that saliva affects our perception and it is also clear that the rate and composition of salivation is dependent on what we perceive. There is a chain of interactions. The aim of this thesis is to show how and to what extent these interactions are present. In order to do this, either the individual response is measured or the individual flow rate and composition is manipulated. The development of the methodology for how this can be done is also part of this work. The thesis is divided into four parts, each resembling one of the interactions between saliva and taste, and saliva and texture.

The aim of the first part (Chapter 2) is to determine the influence of salivary flow and composition on perceived thickness. Food components stimulate salivation, and the flow and composition of the saliva also affect the perception of the food product. In starch-containing foods, salivary α-amylase breaks down the starch and this may cause thinning in semi-solid foods. Can the individual differences in sensory assessment be accounted for by their individual salivary composition? Is it possible to affect the sensory perception of an individual by modifying their salivary flow and composition? This could possibly affect both the texture and the taste perception. If the individual salivary flow rate and α-amylase concentration is modified, the effects on the perceived taste intensity and thickness can be measured. If a big enough group of subjects is used it will also be possible to see how much

these individual differences in physiology account for differences in sensory perception. Vanilla custard was assessed for taste intensity, creaminess and thickness. To extend the range of saliva composition and flow, an α-amylase inhibitor was added to the samples at different concentrations and the pH of the samples was lowered by adding citric acid. From each collected spat-out bolus, temperature, pH, dilution factor and α-amylase activity were measured. Addition of amylase inhibitor reduced saliva α-amylase activity and increased perceived thickness and creaminess. Acidification increased mechanical thickness prior to testing and perceived thickness but did not reduce the *in situ* α-amylase activity because the saliva stimulated by acidified custards was also more concentrated in α-amylase. Alpha-amylase activity varied widely among subjects and therefore a decreased oral α-amylase activity would not guarantee an increase in perceived thickness and creaminess of starch-based foods.

The aim of the second part (Chapter 3) is to investigate the effects of different tastants on parotid salivary flow and composition. The saliva flow and composition affects the perception but different tastes also affect the salivation. Different tastes stimulate different amounts of saliva but do they also affect the saliva composition? Or are the differences in saliva composition caused by the differences in salivary flow rate? In order to test this, the saliva flow and composition needed to be collected and measured continuously so that the salivary composition can be related directly to the salivary flow rate. In this way it can be determined whether compositional differences are caused by the type of tastant or by the salivary flow rate. Five tastants were given in different concentrations in solution and held in the mouth for 10 seconds. The flow rate, protein concentration, and pH of secreted parotid saliva were monitored continuously for 5 minutes. Stimulation by tastants results in an immediate rise in flow followed by a plateau and a rapid return to pre-stimulus flow. The pH increases slowly while the protein concentration decreases slowly, both returning to pre-stimulus levels after about 4 minutes. From a resting flow rate of 140 μL/min, an increase in flow rate to 370 μL/min was caused by stimulation for 10 seconds with 10 mL of solutions of 0.01 M citric acid, 0.13 M $MgSO_4$, 0.25 M monosodium glutamate, 0.5 M NaCl, or 0.5 M sucrose. Comparisons of the different tastants showed that the pH of stimulated parotid saliva increased linearly irrespective of

the nature of the tastant. Though the protein concentration decreased, the absolute protein amount increased together with the total salivary volume. Corrected for the effects of flow rate, the protein amount depended on the nature of the tastant with citric acid stimulating the greatest protein secretion. Flow rate was largely responsible for changes in pH but tastant appears to play an additional role with flow rate on protein secretion.

The aim of the third part (Chapter 4) is to determine the role of saliva flow on the taste perception. Individuals vary largely in their salivary flow and composition and, given the importance of saliva on perception of taste, this might influence how the tastant stimuli are perceived. The tastant needs to be diluted and dissolved by saliva in order to be sensed. Low amounts of saliva, due to old age or illness, therefore often result in a reduced taste sensation. On the other hand individuals with a high salivary flow rate are reported to have a high taste threshold. Can different amounts of saliva, and thus also different dilution factors, affect the taste perception? Furthermore, can taste-taste interactions be explained by an increase in salivary flow rate? In order to answer these questions the amounts of saliva entering the mouth needed to be controlled. In this way the effects of the secreted saliva amounts on the perceived intensity can be controlled. The role of saliva amount on perceived taste intensity can be measured by excluding parotid saliva and adding artificial saliva close to the parotid duct at pre-set flow rates. Significant decreases in perception with increasing salivary flow rates were observed for citric acid and sodium chloride. This can partially be explained by a dilution effect which is in line with previous studies on detectable concentration differences. However, since the bitterness and sweetness remained unaffected by the salivary flow conditions and the dilution effect was comparable to that of saltiness, further explanations are needed. An additional question was whether the suppression of taste intensity in binary mixtures (taste-taste interactions) can be caused by the increased salivary flow rate induced by an additional taste attribute. The results show however that suppression of taste intensity in binary mixtures was not affected by the rate of salivation. Therefore, this is more likely explained by psychophysics.

The aim of the fourth and last part (Chapter 5) is to study texture effects on salivation and the role of saliva on taste-texture interactions. Taste-texture interactions have often been described in the literature, but their nature is not yet fully understood. Taste

perception decreases with increasing thickness of the food item. On the same time chewing stimulates salivation. Is it possible that the increased salivation, induced by the increased thickness, will dilute the tastant and hence decrease the perceived intensity? Or are taste-texture interactions caused by cross-modal interactions? Or is the increased viscosity of the texture decreasing the availability of the taste molecules? In order to answer these questions, a product with both taste and texture attributes was needed in such a way that the taste is not incorporated in the texture and where the taste and texture do not chemically interact. The salivation rate also needs to be measured in order to control for tastant dilution with saliva. If taste-texture effects do occur they can be linked to either to the dilution with saliva or to cross-modal interactions. If taste-texture interactions do not occur they are likely to be caused by a reduced tastant availability. In this study, the taste was separated from the texture and the salivary flow was measured continuously as a response to the manipulation of the sample. In this way the tastant availability could be controlled. Results show that there are no texture effects on the perceived taste intensity. Since no effect was seen in this study set up we draw the conclusion that taste-texture interactions are not caused by dilution effects or cross-modal interactions but can most likely be explained by differences in tastant release.

In this thesis it is shown how the individual perception can be affected by the salivary flow and composition and how the individual salivary flow and composition can be affected by the sensory stimuli taste and texture. In Chapter 6 the overall results are discussed in an integrated manner.

Samenvatting

De perceptie van voedsel wordt beïnvloed door diverse parameters en is afhankelijk van het individu. Wat waargenomen wordt tijdens het eten is daarom niet gelijk voor elk individu omdat iedereen verschillend is. Elk individu heeft verschillen in smaak- en geurreceptoren, verschillen in culturele gebruiken en verschillen in de herinneringen. De factor speeksel voorziet in een additionele bron van variatie, aangezien deze de chemische en structurele compositie van het voedsel verandert gedurende het eetproces. Zowel het volume als de samenstelling van het speeksel varieert sterk tussen verschillende personen en tevens gedurende het eten. Om die reden bepaalt de verdunning en het mengen van het voedsel met speeksel de mate van speeksel-voedsel interactie en daardoor ook de uiteindelijke perceptie van het voedsel.

In welke mate deze parameters de verschillen in perceptie tussen individuen verklaart, is tot op heden niet duidelijk. Het doel van het onderzoek, beschreven in dit proefschrift, is om met name een aspect in meer detail te onderzoeken, namelijk de rol van speeksel. Speeksel productie en samenstelling kan gemeten worden per individu en tevens kan deze beïnvloed worden op een gecontroleerde wijze. Vanuit de literatuur is bekend dat speeksel een invloed heeft op onze perceptie en tevens is heeft de perceptie een invloed op de hoeveelheid en de samenstelling van het speeksel. Er is dus een nauwe interactie tussen voedsel en speeksel. Het doel van het onderzoek beschreven in dit proefschrift is om deze interactie beter te begrijpen en aan te tonen hoe en in welke mate deze interactie optreedt. Om dit te kunnen doen, wordt ofwel de individuele reactie gemeten tijdens consumptie of wordt de individuele hoeveelheid en samenstelling van het speeksel beïnvloed. De ontwikkeling van de methodieken om dit mogelijk te maken vormt een deel van het werk beschreven in dit proefschrift. Het proefschrift is verdeeld in vier delen, waarbij in elk deel een van de interacties onderzocht wordt, namelijk speeksel en smaakwaarneming of speeksel en textuurwaarneming.

Het doel van deel 1 (Hoofdstuk 2) is vast te stellen wat de invloed is van speekselproductie en -compositie op de waargenomen stevigheid van het voedsel. Voedselcomponenten stimuleren de speekselproductie en tevens beïnvloed de speekselproductie en –samenstelling de perceptie van het voedsel. In zetmeel-bevattende voedingsmiddelen zorgt het enzym α-amylase in het speeksel ervoor dat het zetmeel al

afgebroken wordt in de mond, hetgeen kan resulteren in het meer vloeibaar worden van het voedsel. De vraag is daarbij of individuele verschillen in perceptie van dergelijk voedsel verklaard kan worden door verschillen in speeksel samenstelling. Als dat zo is, is het dan mogelijk om de perceptie te beïnvloeden door de speekselproductie en -samenstelling te veranderen? Dit zou dan mogelijk zowel de textuur als de smaakperceptie beïnvloeden. Indien de individuele speekselproductie en tevens de α-amylase concentratie veranderd wordt, dan kunnen de effecten op de waargenomen perceptie van smaak en textuur worden bepaald. Door een groot aantal individuen bij dit onderzoek te betrekken, is het in principe mogelijk om vast te stellen hoe individuele verschillen in fysiologie bijdragen aan de verschillen in de sensorische perceptie. Vanillevla werd in dit onderzoek als testproduct gebruikt en beoordeelt op smaakintensiteit, romigheid en stevigheid. Om de variatie te vergroten in de speekselsamenstelling en -productie werd gebruik gemaakt van een α-amylase remmer die werd toegevoegd aan het product in verschillende concentraties. Bovendien werd de pH van producten verlaagd door citroenzuur toe te voegen. Van de verzamelde monsters speeksel met vla werd de temperatuur, de pH, de verdunningsfactor en de α-amylase activiteit gemeten. Toevoeging van de amylaseremmer reduceerde de α-amylase activiteit in het speeksel en verhoogde de waargenomen stevigheid en romigheid. Verzuring verhoogde de mechanische stevigheid voor het proeven alsook de waargenomen stevigheid, maar niet de *in situ* α-amylase activiteit, omdat het door zuur toegenomen speeksel ook meer *in situ* α-amylase bevatte. Alpha-amylase activiteit bleek sterk te variëren tussen individuen en om die reden betekent een afname van α-amylase activiteit in de mond niet automatisch een verhoging van de waargenomen stevigheid en romigheid van dergelijke zetmeel-bevattende voedingsmiddelen.

Het doel van het tweede deel (Hoofdstuk 3) van dit proefschrift is het onderzoeken van de effecten van verschillende smaakstoffen op de speekselproductie en -samenstelling. De speekselproductie en compositie heeft een invloed op de perceptie, maar verschillende smaakstoffen hebben zelf ook weer een invloed op de speekselproductie. Verschillende smaakstoffen stimuleren verschillende hoeveelheden speeksel, maar hebben ze ook een invloed op de speekselsamenstelling? Of zijn de verschillen in speekselsamenstelling veroorzaakt door de verschillen in speekselproductie? Om hier een beter inzicht in te

krijgen, was het noodzakelijk om de productie en samenstelling van het speeksel continu te bepalen, zodanig dat de samenstelling direct gerelateerd kon worden aan de mate van productie. Op die manier is het mogelijk om vast te stellen of de verschillen in samenstelling veroorzaakt worden door het type smaakstof of door de mate van speekselproductie. Vijf smaakstoffen werden in verschillende concentraties aangeboden aan panelleden en 10 seconden in de mond gehouden. De speekselproductie, de eiwitconcentratie en de pH van het uitgescheiden speeksel werd continu gemeten gedurende 5 minuten. Stimulering door smaakstoffen resulteerde direct in een toename van de speekselproductie, tot een maximum en direct gevolgd tot een afname op het niveau van de productie voordat de stimulus werd gegeven. De pH van het speeksel nam langzaam toe, terwijl de eiwit concentratie langzaam afnam, maar beiden keerden terug naar de oorspronkelijk waarden na ongeveer 4 minuten. Vanuit een rustsituatie van 140 µL/min, werd door stimulatie gedurende 10 seconden met 10 mL oplossing van 0.01 M citroenzuur, 0.13 M $MgSO_4$, 0.25 M natriumglutamaat, 0.5 M NaCl, of 0.5 M sucrose een toename in speekselproductie gemeten tot 370 µL/min. Vergelijking tussen de verschillende smaakstoffen toonde aan dat de pH van het toegenomen speeksel lineair toenam, onafhankelijk van het type smaakstof. En hoewel de eiwitconcentratie afnam in het speeksel, nam de absolute hoeveelheid uiteindelijk wel toe met het totale speekselvolume. Gecorrigeerd voor het effect van de speekseltoename is de eiwithoeveelheid afhankelijk van het type smaakstof met citroenzuur als sterkste stimulator van de eiwitsecretie. De speekselproductie was grotendeels verantwoordelijk voor de verandering in pH, maar het type smaakstof bleek een additionele rol te spelen samen met de speekselproductie op de eiwitsecretie.

Het doel van deel drie van dit proefschrift (Hoofdstuk 4) is om vast te stellen wat de rol is van de speekselproductie op de smaakperceptie. Individuen verschillen in sterke mate in hun speekselproductie en –samenstelling en, gegeven het belang van speeksel op de smaakwaarneming, kan dit een grote invloed hebben op de uiteindelijke perceptie. Smaakstoffen moeten worden opgelost in speeksel om waargenomen te worden. Kleine hoeveelheden speekselproductie, bijvoorbeeld veroorzaakt door ouderdom of ziekte, zullen daarom een lage smaakwaarneming tot gevolg hebben. Aan de andere kant is gevonden dat

individuen met een sterke speekselproductie juist een hoge smaakdrempel hebben. Kan de hoeveelheid speekselproductie, en daarmee de mate van verdunning in de mond, een impact hebben op de smaakwaarneming? En daarbovenop, kunnen smaak-smaak interacties mogelijk verklaard worden door een toename van de speekselproductie? Om dergelijke vragen te kunnen beantwoorden moet de hoeveelheid speeksel die in de mond komt gestuurd kunnen worden. Alleen dan kan het effect van de mate van speekselproductie op de waargenomen smaakintensiteit bepaald worden. Dit laatste kan worden bewerkstelligd door het parotoid speeksel weg te vangen op de plek waar het in de mondholte komt en gelijktijdig kunstmatig speeksel toe te voegen in gecontroleerde hoeveelheden. Door dit te doen werden significante afnames in smaakbeleving waargenomen met toenemende speeksel hoeveelheden voor zowel citroenzuur als zout (NaCl). Dit kan gedeeltelijk worden verklaard door een verdunningseffect dat overeenkomt met eerder onderzoek naar detecteerbare concentratieverschillen. Echter, aangezien de waarneming van bitter en zoet niet beïnvloed werden door de speekselproductie terwijl het verdunningseffect wel vergelijkbaar was met dat van zout, is verder onderzoek noodzakelijk om dit te kunnen verklaren.

Een additionele vraag was of de onderdrukking van smaakintensiteit in binaire mengsels (smaak-smaak interacties) veroorzaakt kunnen worden door een toename van de speekselproductie, geïnduceerd door de additionele smaak component. De resultaten tonen echter dat de onderdrukking van de smaakintensiteit in binaire mengsels niet beïnvloed wordt door de mate van speekselproductie. Om die reden lijkt meer waarschijnlijk dat deze vorm van interactie veroorzaakt wordt door psychofysisch.

Het doel van het vierde en laatste deel (Hoofdstuk 5) is om na te gaan in hoeverre structuur van het voedsel van invloed is op de speekselvorming en op smaak-textuur interacties. Smaak-textuur interacties zijn regelmatig beschreven in de literatuur, maar de aard van deze interacties is nog niet opgehelderd. Smaak perceptie neemt af bij een toename van de stevigheid van het voedingsmiddel. Tegelijkertijd stimuleert kauwen de speekselvorming. Is het mogelijk dat de toename van speekselproductie, gestimuleerd door de toename in stevigheid, ervoor zorgt dat de smaakstof verdund wordt en daardoor de waargenomen smaakintensiteit? Of worden smaak-textuur interacties veroorzaakt door

cross-modale interacties? Of is het zo dat de toegenomen viscositeit (stroperigheid) van de structuur ervoor zorgt dat er een verminderde beschikbaarheid is van smaakstoffen? Om deze vragen te kunnen beantwoorden, was het nodig om een product ter beschikking te hebben met zowel smaak als textuur attributen, en wel op zodanige wijze dat de smaakstoffen niet in de structuur geïncorporeerd zijn en waarbij de smaakstoffen ook niet reageren met de structuur van het product. Tevens was het noodzakelijk om de speekselproductie te kunnen meten, om zodoende de mate van verdunning door speeksel te kunnen sturen. Als smaak-textuur effecten optreden, dan kunnen ze ofwel verklaard worden door aan de mate van verdunning door het speeksel of door cross-modale interacties. Indien onder dergelijke omstandigheden er geen smaak-textuur interacties optreden, dan is het aannemelijk dat ze normaliter veroorzaakt worden door verminderde beschikbaarheid uit voedingsmiddelen met een hogere stevigheid. In dit hoofdstuk werd de smaakstof gescheiden van de textuur en werd de speekselproductie continu gemeten als een reactie op de manipulatie van het product in de mond. Op deze manier kon de beschikbaarheid van de smaakstof gestuurd worden. De verkregen resultaten tonen dat er onder deze omstandigheden geen effect is van de textuur op de waargenomen smaakintensiteit. Aangezien er geen effect werd gevonden met behulp van deze onderzoeksopzet, werd de conclusie getrokken dat smaak-textuur interacties niet worden veroorzaakt door verdunningseffecten of cross-modale interacties, maar hoogst waarschijnlijk verklaard kunnen worden door verschillen in het vrijkomen van de smaakstoffen uit het voedsel.

Dit proefschrift laat zien hoe individuele smaakwaarnemingen worden beïnvloed door de speekselproductie en –samenstelling en hoe de individuele speekselvorming beïnvloed kan worden door sensorische stimuli en textuur. In Hoofdstuk 6 worden de resultaten uit de verschillende hoofdstukken geïntegreerd en bediscussieerd.

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‘Think how really precious is the time you have to spend, whether it is at work or with your family – every minute should be enjoyed and savoured.’ *Earl Nightingale*

List of publications

Heinzerling, C. I., Stieger, M., Bult, J. H. F., Smit, G. Taste-texture interactions: tastant release, impact of salivation or perceptual interactions, submitted.

Heinzerling, C. I., Stieger, M., Bult, J. H. F. and Smit, G. (2011), Individually modified saliva delivery changes the perceived intensity of saltiness and sourness, *Chem. Percept.* 4(4): 145-153.

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Chen, H. J., **Karlsson, C.** and Povey, M. J. W. (2005), Acoustic envelop detector for crispness assessment of biscuits, *J. Text. Stud.* 36(2): 139-156.

Curriculum vitae

Cathrine Heinzerling was born on the 10th of June 1981 in Uppsala, Sweden as Cathrine Karlsson. In August 2001 she started studying to become a Food Engineer at the University of Agricultural Sciences in Skara. During her studies she carried out three research projects; minimization of economic loss caused by water loss in meat products (1), developing of a biosensor to monitor the temperature of food packages during transport (2), and development of an acoustic detector for determining crispness and crunchiness in food products (3). For the second research project she was awarded with the LLK-award from the Swedish Association for Quality, section Food, Pharmaceuticals and Chemistry (SFK-LLK). The third research project was carried out at the Procter Department of Food Science in Leeds. For this work she obtained both an ERASMUS-grant and a grant from the Swedish University of Agricultural Sciences. After obtaining her degree as Food Engineer in June 2004, she continued her studies at the University of Leeds where she further developed the acoustic detector. She also worked at understanding the sources of sound during structural break up and its relation to the human perception of crispness. She obtained her MSc in Food Science in 2005. Her studies were sponsored from Stable Micro Systems. In October 2005 Cathrine started her PhD at Wageningen University in collaboration with TI Food and Nutrition. In 2007 she was awarded the Silver poster award from the 5th NIZO Dairy Conference, a new investigator presentation competition for her research and presentations. The aim of her PhD research, presented in this thesis, was to map the interactions between saliva and perception of texture and taste. Since January 2012 Cathrine works as a Senior Clinical Supply Project Manager at Abbott in Ludwigshafen, Germany.

Overview of completed training activities

Discipline specific activities

VLAG courses
Advanced course: Food perception and preference (2005)
Regulation of food intake (2006)

Courses and conferences
Speaker at the International ultrasonic conference, Leeds Foodchain (2006)
Diogenes conference, Potsdam (2006)
Poster presentation at the Diogenes conference, Mallorca (2006)
Poster and oral presentation at the NIZO Dairy conference (2007)
Symposia including presentation at Unilever (2007 and 2008)
Poster presentation at the Unilever conference (2008)
Poster presentation at ECRO (2008)
Poster presentation at Pangborn (2009)
Symposium including presentation at DSM (2009)

General courses

Debating course from WCFS (2006)
PhD competence Assessment from WGS (2006)
MBTI, Myers-Briggs Type Indicator, Leuwendaal (2008)
Career perspective course from WGS (2009)

Additional activities

Preparation of the PhD research proposal (2005)
TIFN, program 2 meetings (2005-2010)
Food Chemistry PhD trip to Belgium, France and England (2006)
Organisation of international excursion (2006)

The studies presented in this thesis were performed within the framework of TI Food and Nutrition.

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