

A ROUGH GUIDE TO TEXTURE

ORAL PHYSIOLOGY AND TEXTURE
PERCEPTION OF SEMI-SOLIDS

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ORAL PHYSIOLOGY AND TEXTURE PERCEPTION OF SEMI-SOLIDS

EEN LEIDRAAD NAAR TEXTUUR
ORALE FYSIOLOGIE EN TEXTUURPERCEPTIE VAN HALFZACHT VOEDSEL
(Met een samenvatting in het Nederlands)

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(Med en sammanfattning på svenska)

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CHAPTER 1
INTRODUCTION

TEXTURE

Sensation and perception

Sensation is the receptor response to bodily stimulation, whereas perception, as defined in the Oxford dictionary, is the awareness through the senses interpreted in the light of experience. The senses are touch, including temperature, in addition to taste, smell, hearing and sight. Perception can be the awareness arising through one single sense or through a combination of many. Perception of food is the result of food characteristics interacting with the processes in the mouth, as interpreted by the brain.

We eat several times a day and most of the time we are actively aware of what we eat. The food undergoes many events on its way from the plate to the stomach: e.g. spooning, stirring, ingestion, mastication and swallowing. In the oral cavity, the food is subjected to several mechanical and chemical processes: It is chewed and otherwise manipulated mechanically, e.g. by the tongue. Furthermore it is diluted and broken down by saliva, heated or cooled by the ambient temperature of the mouth, formed into a bolus and finally swallowed. The numerous receptors in the oral cavity and nose respond to the initially ingested food and monitor the changes during processing. This leads to central perceptions of taste, odour, irritation and texture of the food.

What is texture?

In literature, a number of definitions of texture can be found. One of the most used definitions was stated by Szczesniak (1), who defined texture as “the sensory manifestation of the structure of the food and the manner in which this structure reacts to the applied forces, the specific senses involved being vision, kinesthesia, and hearing”. Jowitt (2) extended the definition of texture: “Texture is the attribute of a substance resulting from a combination of physical properties and perceived by the senses of touch (including kinesthesia and mouthfeel), sight, and hearing. Physical properties may include size, shape, number, nature and conformation of constituent structural elements”. Jowitt also stated that the appreciation of texture involves the subtle interaction between both motor and sensory components of the masticatory and the central nervous system. In the study presented in this thesis, I have chosen to use Jowitt’s definition as the working definition.

When asking lay people to describe food, taste and flavour are most often mentioned. However, subconsciously, texture of food is of great importance for the appreciation of food. Just think of soggy cornflakes, water thin chocolate mousse or wilted lettuce. Conversely, very good texture, such as a soft and airy hollandaise sauce, is associated with excellent cooks. Texture is not only important for the appreciation, but also for the recognition of food. After blending food products, the lack of texture cues resulted in only 40% of the products being correctly identified from their flavour only (3).

Food texture and its importance to the consumer are considerably less well understood than factors such as odour and taste (4). In contrast to odour or taste, there are no specific

receptors for texture *per se*. Texture perception has in the past received relatively little research attention compared with odour and taste. However, this is a changing trend as reported by Szczesniak (5).

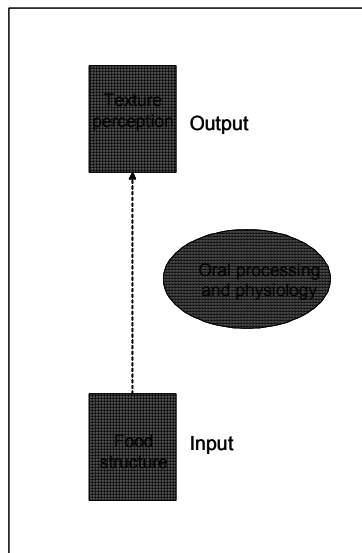


Fig 1. Oral physiology as the missing link in the relationship between food structure (input) and texture perception (output).

Missing link in understanding texture

Previous research on food texture has focused mainly on rheological measurements, frequently correlated with sensory data of the same product (Fig 1). Conventional rheological measurements, such as viscosity and puncture tests, are not based on oral systems, but have their origin in process technique and product control (6). As a result, rheological measurements have often turned out to be unsatisfactory in explaining the relationship between food structure and texture perception. This could be explained by the notion that this approach disregards the oral processing and physiology of the mouth (7). Moreover, most sensations associated with food texture occur only when the food is manipulated, deformed, or moved across the oral receptors. During the time in the mouth, the stimulus undergoes constant changes: it is heated or cooled; diluted and broken down by saliva; and manipulated mechanically. This makes the mouth a very challenging system to mimic *in vitro*.

Another indication to that oral processes are important, is that human volunteers (subjects) assessing the same stimulus do not only differ largely in their ratings of that stimulus, the oral physiological parameters also exhibit large inter-individual variations. In this light, oral physiology, e.g. oral processes (manipulation, mixing and dilution of food in the mouth) and oral sensitivity and receptors, possibly is the “missing link” in understanding the relationship between food structure and texture perception (Fig 1).

THE PRESENT STUDY

Factors influencing texture

There are numerous factors, both product and subject related, that can influence texture perception (Fig 2.). These factors can affect texture perception directly or indirectly. Many of the factors influence each other, which makes the whole concept rather complex. Since there are many possible interactions, no lines have been added in the diagram, indicating that all interactions are possible. This diagram is not exhaustive, but includes a collection of factors for food types ranging from solid to liquid.

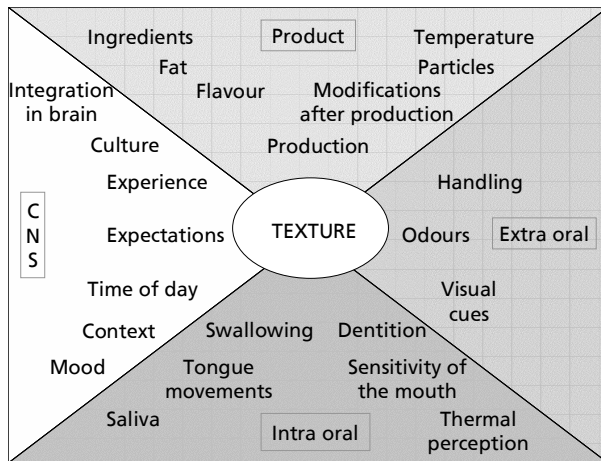


Fig 2. Diagram of factors that can influence food texture

Starting at 11 o'clock product ingredients (top left in Fig 2) are major determinants of the product structure. The ingredients include thickeners, type of starch, oil, water etc. Moving clockwise, the level of fat is thought to influence flavour release, mouth feel, and thermal perception. In addition, the ingredients can affect the flavours e.g. by having off-flavours. Product structure is of importance for how the product will be handled in the mouth. Production techniques, e.g. homogenization, baking and

freezing affect the final structure strongly. Various matters, such as particles, can be added after production, to modify the product. Temperature of the product does not only affect the structure, it can also influence the perception of food texture, flavour and irritation.

Texture is also perceived outside the mouth (extra oral). Before the food enters the mouth, visual cues such as colour, shine, grains, and heterogeneity (lumps), provide information on the texture of the food. Additional information can be obtained by handling the food, e.g. stirring, spooning and cutting.

Intra-oral factors that are subject-related can affect the food itself and how it is perceived. These are: thermal perception; sensitivity of the mouth to touch and size; dentition; swallowing; movements of the tongue in relation to the palate; and saliva amount and composition.

Finally, the central nervous system is an important determinant in texture perception. Memory and emotional state of the person eating the food, social background, time of day and expectations could be of importance. During exposure to different foods, the perception and appreciation of food will change due to experience. In different cultures, different textures are favourable, such as stickiness and pliability in Japan (8).

A selection of factors, potentially influencing texture, was made to study further. Since the research was aimed at investigating the role of oral physiology on texture perception, mainly factors that are subject-related and applicable to semi-solids were selected: Saliva, sensitivity, added particles, tongue movements and temperature.



This selection is in accordance with Szczesniak's ideas (9). She stressed that tactile perception, perception related to size and position of particles in the mouth and temperature are very important.

Multi-disciplinary project

The research presented in this thesis is part of a multi-disciplinary project with the scope of investigating the fundamentals of texture of semi-solids. The relation between food structure and texture perception was investigated by the combined efforts of three disciplines; sensory science, oral physiology and physical science (rheology).

Semi-solids were chosen as stimuli, as they can be easily and reproducibly modified. The independent variation in starch content, starch type, fat etc. resulted in a large variation of stimuli. In addition, the choice of semi-solids largely excluded the effects of the chewing process and teeth, which enabled the research to focus on other oral mechanisms. Oral texture attributes of semi-solids can be divided into functional sub-groups: lip-tooth feel, mouth feel and after feel (10).

Subjects and individual differences

Human perception of texture is a physical and psychological response to a stimulus, thus a full description of texture can be achieved only by the employment of human volunteers. Previous research has shown that there are large differences in reported sensations among subjects, even though they are assessing the same product. In part these differences could be a result of physiological differences between individuals, in part they reflect differences in the use of the measurement scale and terminology. In this research we have focused on investigating the physiological differences among subjects.

Healthy adult volunteers were screened for well functioning smell and taste. The selected subjects were trained in QDA (Quantitative Descriptive Analysis) and formed a weekly panel.

Aim of the research

The previous sections discussed the complexity of understanding texture and the involved factors. This includes the difficulties of relating conventional rheology with texture perception and of oral physiology as possibly being the missing link. Further, it has been suggested that the differences in perception among subjects might partly be explained by differences in their oral physiology.

The aim of this research was to examine the role of oral physiological processes on oral texture perception of semi-solids and to investigate whether individual differences in perception could be attributed to and explained by differences in oral physiology among subjects.

The next two sections offer a general overview of a few aspects of physiology that are referred to in subsequent chapters of this thesis.

SALIVA

Secretion of saliva

Human whole saliva consists of the combined secretions from the salivary glands, and its characteristics are dependant on the origin of the secretion. Whole saliva is derived mainly from the three paired major salivary glands – the parotid, submandibular, and the sublingual glands (Fig 3). They are characterized by the presence of a large number of secretory cells. In addition to these, minor salivary glands are dispersed throughout the mouth, including the palate, lips, cheeks and tongue. The parotid gland, the largest of the salivary glands, is a purely serous gland that produces watery, enzyme-rich saliva upon stimulation which is rheologically speaking comparable to water (11).

Parotid saliva is virtually absent during sleep, but can easily be stimulated to be the major constituent of whole saliva. The parotid gland contributes up to 50% or more of the stimulated saliva in the mouth. During sleep and rest whole saliva consists for 70% of submandibular saliva, whereas during stimulation this decreases to 30-45 % (12). The submandibular glands are mixed glands, containing both serous and mucous cells, which secrete a more viscous mucus-containing saliva. The sublingual glands consist mainly of mucous cells. As a result, sublingual saliva is very viscous, which can be attributed to the high levels of mucins present. The minor salivary glands contribute to saliva volume with 7-14% (13) and the secretion contains high levels of protein, such as immunoglobulins (12).

The mean total amount of saliva secreted per day is estimated to be between 500 and 1500 ml. Watanabe and Dawes estimate was about 570ml (14). This calculation implies 54 minutes of eating (4 ml/min), 16 hours of awake activities (0.3 ml/min), and 7 hours of sleep (0.1 ml/min) (15). Hence, the flow rates exhibit circadian fluctuations (16;17), and depend largely on the activity and type of stimulation. Normally, mean saliva flow at rest is around 0.3ml/min, whereas during stimulation, the flow can increase to a maximum of 7ml/min (18). Despite the large variation in normal salivary flow rates, it is generally agreed that salivary flow rates of 0.1ml/min or less (unstimulated) and 0.5ml/min or less (stimulated) are abnormally low (19).

Salivation can be stimulated in various ways: mechanical input mediated by oral mechanoreceptors (20) and taste, where acids are the most, and sugars the least potent stimulators, represent the major input. Olfaction, the sight of food and thermal stimulation are

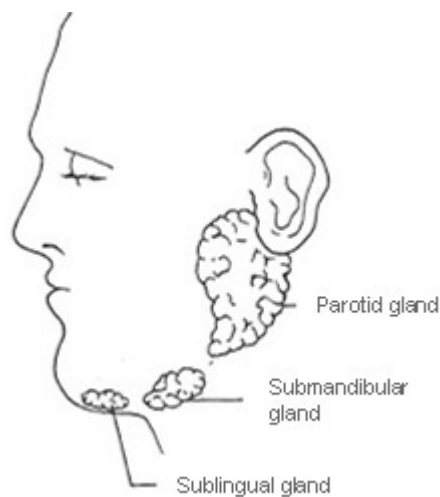


Fig 3. The location of the three major salivary glands.



other inputs that contribute to salivation (20;21). Factors such as mood, disease, medication, body hydration and exercise (22) also affect salivary flow and composition. The salivary glands are innervated by both parasympathetic and sympathetic nerve fibres (23). Parasympathetic stimulation increases the synthesis and secretion of amylase, mucins and saliva. Sympathetic stimulation on the other hand causes constriction of blood vessels, with consequent reductions in salivary flow from the gland (23).

Constituents and actions of saliva

Saliva consists for more than 99% of water and the remaining 1 % contains a large number of organic and inorganic constituents (24;25). Saliva contains minerals, enzymes e.g. α -amylase, a large number of proteins, such as proline rich proteins (PRPs), and mucins, glycoproteins with a number of functions, e.g. lubrication and antibacterial actions. A number of these constituents of saliva, including water affect the structure and perhaps also the perception of food.

Saliva is indeed expected to be involved in our perception of the taste, flavour and texture of foods. The effects of saliva on food leading to changes in perception are plentiful. Mixing of saliva with food can have a diluting effect (26;27) and play a role by initial breakdown of food (28;29;29), by affecting flavour release (26;30-33), transport of taste compounds to the taste buds (30-33), precipitation of proteins by tannins e.g. resulting in a sensation of astringency (34;35), and acting as a buffering system (36-38), affecting the degree to which we perceive sourness (39). In addition, the large salivary proteins can influence the lubrication (12) and hence perhaps the perception of attributes such as smoothness and astringency (35;40) and facilitating manipulation of food in the oral cavity and swallowing. These examples indicate the value of saliva for the appreciation and acceptance of food.

Amylases are enzymes that catalyze the hydrolysis of starch into smaller carbohydrate molecules such as maltose and glucose. There are two types of amylases, denoted alpha and beta, that differ in the location they attack the bonds of the starch molecules. By hydrolyzing the starch of semi-solids, such as custard desserts, into sugar molecules, the starch loses its ability to bind water, resulting in a decrease in product viscosity.

Since saliva is always present in the mouth, with increasing amounts during eating or otherwise stimulated, we hypothesized that saliva would be important for the sensation and perception of semi-solids. We therefore investigated both the amount and composition of saliva in the subjects and related these to their perception of the foods in order to establish the importance of saliva on perception of semi-solids.

RECEPTORS AND SENSATIONS

The studies included in this thesis, have only paid little attention to the receptors and processing of the signals in the central nervous system. Yet, sensation and perception are a result of receptor signals and central processing. Therefore, the following section gives an overview of the oral receptors and how the signals are conveyed and processed to give the resulting perception.

Humans have four classes of receptors, each of which is sensitive primarily to one type of physical energy – chemical, mechanical, thermal and electromagnetic. In the mouth all types, except the photoreceptors sensitive to electromagnetic energy, are present. The chemical receptors include taste and smell; the mechanoreceptors mediate sensations of touch and proprioception; the thermoreceptors sense the temperature of the body and objects that we come in contact with and nociceptors signal sensations of pain. All these types of receptors contribute to the total sensation and perception of food that we ingest.

Taste and smell

The senses of taste and smell have been studied extensively and much is known on how tastes, odours and flavours are sensed and how the sensations are processed in the central nervous system. Food is often classified on the basis of their taste and smell.

Taste

Chemical constituents of food interact with receptors on taste cells, which are found in taste buds distributed throughout the oral cavity, pharynx and upper part of the oesophagus. Most taste stimuli are hydrophilic molecules that are soluble in saliva. The gustatory system distinguishes four basic stimulus qualities: salt, sweet, sour, and bitter. Monosodium glutamate may represent a fifth stimulus category, called umami. Recent evidence indicates that fat may represent an additional taste quality (41). These tastes can interact, to enhance or suppress the perception.

Smell

The olfactory system reacts to airborne stimuli, called odorants. These interact with olfactory receptor neurons in the nasal mucosa located in the roof of the nasal cavity (42). From the olfactory receptors neurons, numerous olfactory cilia, which are in fact the structures in contact with the odorants, protrude into the layer of mucous in the nasal lumen. The odorants can reach the nasal cavity through the nose (orthonasal) or through the mouth (retronasal), which is the case when eating a product. In the nasal lumen the odorants bind to specific receptors on the cilia and a signal is transduced to the olfactory bulb and then further on to the olfactory cortex.



The somatic sensory system

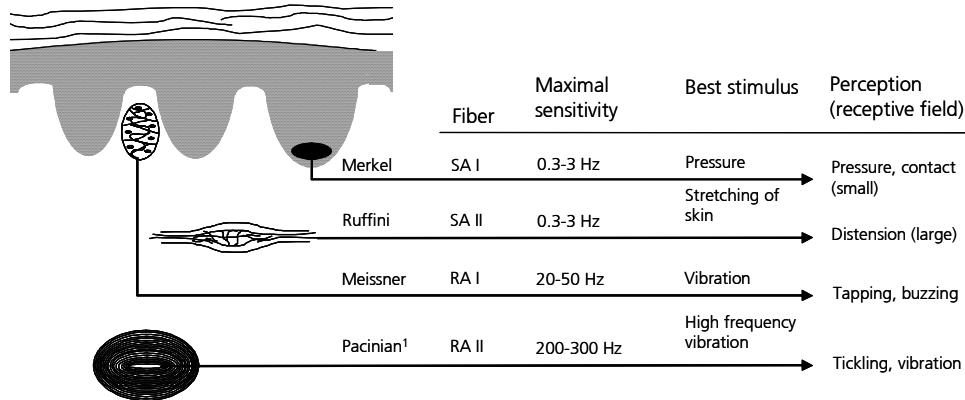
The mouth is a very sensitive organ. The oral cavity is one of the regions of the body most densely innervated with nerve fibres and receptors (43) and is exquisitely sensitive to tactile stimulation (44;45). This means that thresholds for somesthetic stimuli are lower and discrimination is better than on most other skin areas of the body. Thresholds for detection of light touch are lowest on the tip of the tongue and hard palate (44;46). Somesthetic receptors are found in all regions of the oral cavity, including the lips, tongue, teeth and mucosa.

The somatic sensory system transmits information about four modalities: touch, temperature, pain and proprioception. The receptors for each modality are specialized structures, allowing them to sense specific types of stimuli. Cutaneous receptors can be subdivided according to the type of stimulus to which they respond. The major types of receptors include mechanoreceptors responding to tactile stimuli, thermoreceptors and nociceptors, responding to pain.

Mechanoreceptors in the mouth

Mechanoreceptors respond to tactile stimuli, such as pressure or tapping. There are a number of different types of receptors present in the skin of humans. Four major types of histological nerve endings (Merkel disks, Ruffini endings, Meissner corpuscles and Pacinian corpuscles) are associated with a particular type of tactile perception: pressure, stretch of skin, taps on skin, and high frequency vibration. However, in the mouth and specifically on the tongue, there is as yet only little information on the morphology of the nerve endings (personal communication, Mats Trulsson).

However, functionally, the receptors behave similarly in all areas and the ones present in the mouth and lip closely resemble the mechanoreceptors previously described for the skin of the hand. These are slowly adapting type I and II (SA I and SA II) and rapidly adapting type I (RA I). No RA II afferents have been found in face/mouth, i.e. no receptors showing response properties similar to Pacinian-corporuscle afferents were observed. Barlow (47) concludes that pacinian-type frequency sensitivity characteristics of the finger, was absent in the face. The various receptors are sensitive to different frequencies of vibration, ranging from 0.4 Hz to over 500 Hz. A summary is presented in Fig 4.



The majority of the mechanoreceptive afferent units in the skin of the human face are slowly

Fig 4. Summary of nerve endings, fibers and perception of skin.

¹ not present in the oral cavity

adapting with small and well defined receptive fields (48). This makes these receptors very well suited for resolving fine details (49). Johansson *et al.* (48) found primarily slowly adapting units in the oral mucosa and the transitional zone of the lip. In contrast, the tongue has primarily rapidly adapting receptors (50). Receptors associated with rapidly adapting fibres notice changes, they respond only to the application and removal of a stimulus. In contrast, slowly adapting receptors respond to prolonged and constant stimulation, and, hence are well suited for signalling the location of stimulation and fine details. The receptors respond best to frequencies within a certain range. However, if the stimulus is well above threshold, a number of receptors can be activated at once (49).

Mechanoreceptors in the mouth are not yet fully understood. In the sensation and perception of oral texture, the tactile stimuli are probably the most prominent clues to texture. Hence, a deeper insight into the mechanoreceptors and their exact function in food sensation would be of great importance in this area of research. This could be one way to proceed to gain more fundamental knowledge of the origins of oral texture sensations.

Thermoreceptors

Thermal sensations result from differences between temperature of the air or of objects contacting the body and the normal skin temperature. There are two types of thermoreceptors in the skin, responding to specific temperatures and changes in temperature: cold and warm receptors. Both classes are slowly adapting, although they also discharge phasically when skin temperature is changing rapidly. The receptors are active over a broad range of temperatures – cold: 20°C - 40°C; warm: 30°C - 48°C (49). At moderate skin temperatures, such as 35°C, both types of receptors may be active. However, as the skin is warmed, the cold receptors stop firing and conversely, as the skin is cooled, the warm receptors become inactive. Cold and warm receptors also stop firing altogether as the temperature extends into the noxious



(damaging) range (below 5°C and above 50°C) (51). At these stimuli temperatures, humans perceive freeze and heat pain rather than sensations of cold and warmth. The fact that the face and particularly the lips contain more temperature-sensitive spots than any other region of the body, suggests that the temperature of the food entering the mouth is well sensed. This could have an effect on the way the food is perceived. Oral parts can be heated and cooled down depending on the temperature of the food, which in turn also physically affects the food.

Nociceptors

The sensation of pain serves an important protective function: It warns of injury that should be avoided or treated. Pain is mediated by specialized free nerve endings, called nociceptors. They respond to stimuli that may produce tissue damage, such as intense pressure, extreme temperature, or burning chemicals. This response can be direct to some noxious stimuli and indirect to others by means of chemicals released from cells in the traumatized tissue (51). There are three major classes of cutaneous nociceptors that often work together: the A δ mechanical and thermal nociceptors, and the C-polymodal nociceptors, that respond to noxious stimulation of varying origin. The fast sharp pain is transmitted by the A δ fibres and the slow dull pain by the C fibres (52). Unlike the specialized somatosensory receptors for touch and pressure, most nociceptors are free nerve endings.

Proprioceptors

Proprioception is the sense of static position and movement of the limbs and body. There are two sub-modalities of proprioception: the sense of stationary position of the limbs and the sense of limb movement. Cutaneous proprioception in the face is especially important for control of lip movement in speech and face expressions (51). Three types of receptors in muscle and joints transmit proprioceptive information: Muscle spindles are situated in the muscles and signal changes in the length of muscles, Golgi tendon organs signal changes in tension, and receptors located in joint capsules sense flexion or extension of the joint(51;53;54).

Periodontal receptors

Human teeth are sensitive to very small forces applied to them (55). Teeth are attached to the alveolar bone by the periodontal ligament. This ligament is invaded by nerve fibres terminating in periodontal mechanoreceptors, which respond to loading of the teeth and which are dependent on the direction in which the forces are applied (56). The periodontal receptors probably especially play a role for solid and hard foods (57).

From sensation to perception

The oral regions are innervated by afferent nerve fibres in the trigeminal nerve (cranial nerve V). Hence, tactile information from the receptors in the mouth is conveyed to the central nervous system by the trigeminal somatic sensory system (Fig 5). The oral receptors initiate action potentials upon stimulation. This activity is conveyed via first-order neurons in the trigeminal ganglia, entering the brain stem at the level of the pons, further on to second-order neurons in the trigeminal brainstem complex. This complex has two major components: the principal nucleus (responsible for processing mechanosensory stimuli) and the spinal nucleus (responsible for painful and thermal stimuli). The second-order neurons of the trigeminal brainstem nuclei give off axons that cross the midline and ascend to third-order neurons in the ¹VPM nucleus of the thalamus. The axons arising from neurons in the ²VP complex of the thalamus project mainly to cortical neurons located in the primary

somatosensory cortex (SI, also known as Brodmann's area). Somatic sensory information is distributed from the SI to "higher-order" cortical fields, such as the adjacent secondary somatosensory cortex, which sends projections to limbic structures, e.g. the amygdala and hippocampus. On all levels neurons also receive parallel information. The representations from each modality (taste, vision, olfaction and touch) are brought together in multimodal regions, such as the orbitofrontal cortex (58). The signals are integrated to a complete picture, the perception.

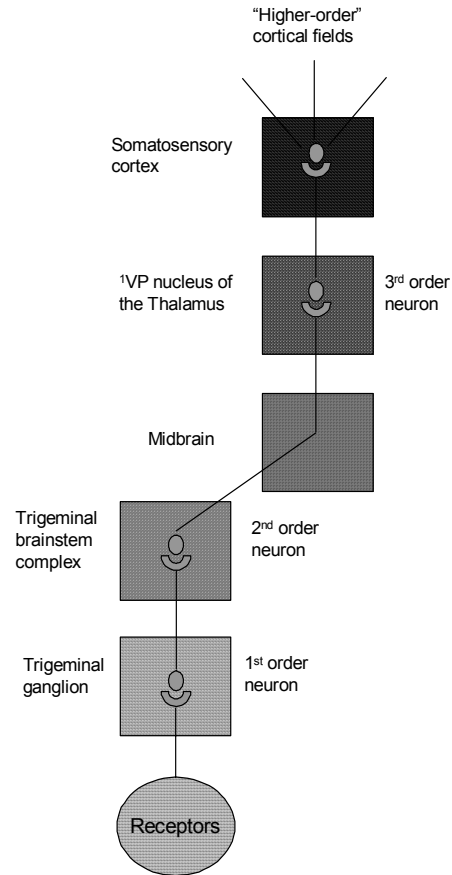


Fig 5. Trigeminal pathway from receptor to higher brain centres.

¹ Ventral Posterior Medial Nucleus of thalamus

² Ventral Posterior complex of thalamus

 OUTLINE OF THE THESIS

This thesis presents eleven studies on four aspects of oral physiology in relation to texture perception (Fig 6):

- Oral sensitivity and particles (chapter 2-5)
- Manipulations of tongue movements (chapter 6)
- Oral and product temperature (chapter 7-8)
- Amount and composition of saliva (chapter 9-12)

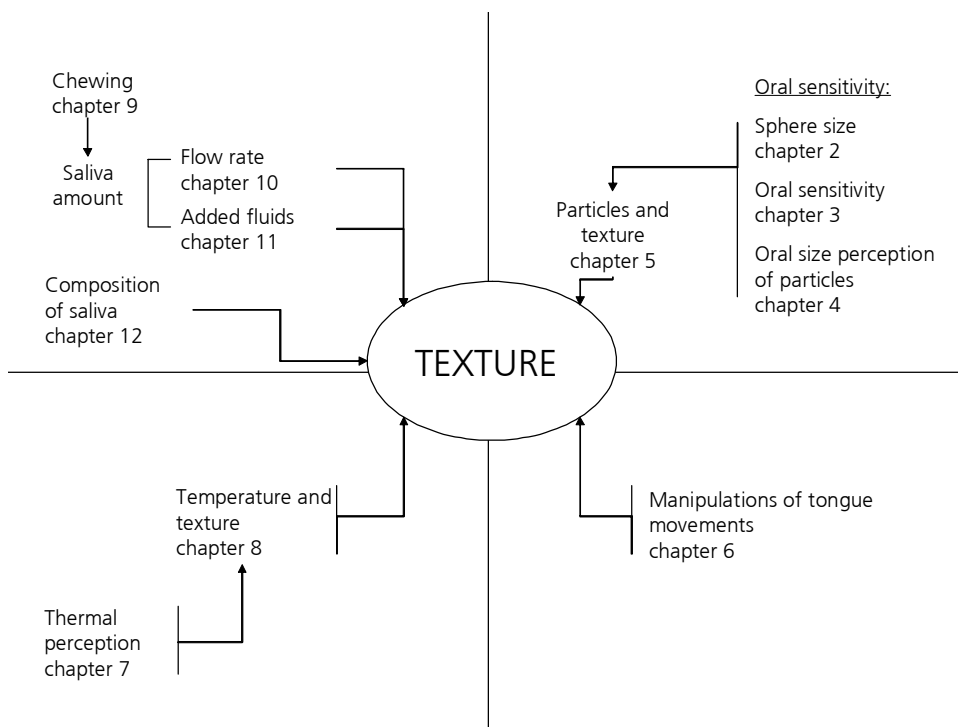


Fig 6. Schematic representation of the thesis outline. The research topics are depicted in relation to texture

Oral sensitivity and particles

Sensitivity of the mouth includes the ability to assess shape, size, and surface texture. Information on the significance of the various oral parts in oral size perception and sensitivity is required to understand their role in the control of bite size and swallowing, and perception of food. Practically all food contains particles. It has been suggested that the presence of particles in food may affect the perception of sensory attributes. While some are obviously present such as pits in berries, others are small, or soft and hardly noticeable, such as oil

droplets in mayonnaise. Large particles in low concentrations are likely to be perceived as separate entities, e.g. seeds in a watermelon. Conversely, small particles of high concentrations are more likely not to be noticed separately, but instead to have an effect on the texture of the product, e.g. graininess.

Imai et al. have studied grittiness in the mouth (59-61). Also other types of texture were studied, e.g. digital roughness when moving the fingers over an embossed surface (62;63). For oral perception of grittiness, it has been reported that concentration, size and shape of the particle are of importance, as well as the medium in which they are dispersed (59;61;64). This thesis presents four studies that address these aspects of oral sensitivity and the effect of particles on texture perception.

In **chapter two**, the perception of sphere sizes (4-9 mm), and the relative importance of tongue and palate in size perception were addressed. To investigate the mechanisms of particles sensed separately, it was chosen to take things to extremes and an experimental set-up was chosen, in which spheres were used which could be handled separately and safely. Subjects are to some degree able to detect and measure the size of objects in the mouth. This study questioned whether this is done by assessing the weight or the volume of the object in the mouth and what the most important oral parts included in this assessment are.

Chapter three: Oral sensitivity has often been measured to track damage and rehabilitation after occasions of stroke (65), prosthodontic treatment (66;67), and speech disorders (68). Various methods to measure oral sensitivity have been employed, including oral form recognition (66;69-72), interdental size and weight discrimination (73), intra-oral size judgements of small holes (74-77), cylinders (78), liquid volume during swallowing (79), and 2-point discrimination (44). The study presented in chapter 3 investigates the relation between three different measures of oral sensitivity to size, i.e. chewing thresholds, two-point discrimination and size perception of spheres. In addition, the importance of the tongue and palate in oral sensitivity and size perception was investigated by applying local anaesthesia.

Chapter four addresses how oral size perception is affected by different types of particles in sizes varying from 2-230 μm and media of different viscosities. Two different methods of assessing size (direct scaling and forced ranking) were compared.

Chapter five. Following the results of the previous studies (chapter 2-4), the next step was to investigate the effect of added particles, including the effect of particle size on texture perception. In addition, the relation between subjects' assessment of particle size, and their perception of texture in custard dessert was studied.



Manipulations of tongue movements

In **chapter six**, a new approach to gathering data on the relation between oral movements and attributes was explored. Oral movements were experimentally modified and their effects on flavour, mouth- and after-feel sensations evaluated. To gain insight into the effect of oral processes on perception, we defined a set of 5 specific oral manipulations and investigated their effects on the perception of semi-solid foodstuffs. Modifications of tongue movements ranged from simply placing the stimulus on the tip of the tongue to vigorously moving it around in the mouth.

Oral and product temperature

Thermal effects on texture perception can be mediated by physico/chemical changes in the product, or by differences at the level of the mucosa. Product temperature could influence the viscosity of the product and the ratio of solid and melted fat and thereby influence the quality and the thickness of the oral coating formed. Foods, initially at temperatures higher or lower than body temperature, undergo physical changes when eaten as thermal equilibrium occurs. The differences in oral temperature could affect receptor response, blood flow and have a secondary effect by altering the product on contact, all of which may change the response to the stimuli. If oral temperature is important, it can be hypothesized that heating or cooling the mouth can modify sensory ratings.

Chapter seven reports on the effect of oral and stimulus temperature on thermal perception.

In **chapter eight**, the effects of oral and product temperature on sensory perception are studied.

Amount and composition of saliva

Saliva is always present in the mouth and the amounts increase during eating. The food is mixed and diluted and break down is initiated by saliva. It seems likely that the amount of saliva present in the mouth during mastication could affect the perception of the food. In addition, the composition of saliva varies largely between subjects and depends on the type of stimulation. Hence the composition of saliva might have an effect on the actual physical structure of food and on the interaction between the food and the mucosa.

Chapter nine reports on a study in which the individual salivary flow rates at rest and after different stimulations are correlated with the subjects' sensory ratings.

Chapter ten: The effect of an artificial increase in amount of saliva and fluid in the mouth during eating were studied and an attempt to separate the action of the different liquid components of saliva was made.

The subjects' individual composition of saliva was analyzed in **chapter eleven** and the correlations between the salivary components and sensory perception determined.

In **chapter twelve**, the salivary flow was measured during chewing on parafilm and a number of different foods. We also determined the duration of a chewing cycle, the number of chewing cycles until swallowing, and the time until swallowing for these foods. The relations among these parameters were examined.

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CHAPTER 2

THE INFLUENCE OF DENSITY AND MATERIAL
ON ORAL PERCEPTION OF BALL SIZE
WITH AND WITHOUT PALATAL COVERAGE

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ABSTRACT

The size of a bolus determines how it will be manipulated in the mouth and swallowed.

Ten healthy individuals assessed the size of ball bearings of five sizes (4-11 mm diameter) and four materials with different densities in order to investigate the effect of weight on the oral size perception. To study the role of the tongue and palate, the experiment was performed with and without a custom-made plastic palate.

The results revealed that size itself determines the size perception, and that material and weight are negligible factors. An illusional effect in the direction of under-estimation was found for the ball bearings, for the small sizes up to 8 mm diameter. While wearing a plastic palate a significant improvement ($p < 0.05$) occurred; the subjects performed better and there was less under-estimation. An explanation for this could be that only a minor part of the total area of the ball bearing touches the palate and is hence detected, while the tongue alone is more compliant and thereby able to sense the ball's whole size.

INTRODUCTION

The perception of size in the mouth is of interest because the size of the initial bolus and fragmented particles in the food determine the number of chews, swallows, and the amount of saliva required for breakdown and bolus formation during mastication. The type of food and the size of the bite also determine the degree of manipulation and the complex movements of the tongue, oropharynx and larynx involved in deglutition.

Bite size appears to be under careful control and affects the rate of ingestion (1), with a small bite size resulting in a low ingestion rate. Although the volume of food ingested at a single bite varies both between individuals and between foods, for any one experimental participant consuming a specified food the bite size is consistent; this implies that the subject in some way is able to detect and measure the size of the bite in the mouth. Is this done by assessing the weight or the volume of the food, and which are the most important oral parts included in this assessment?

The sensory and perceptual characteristics of the mouth have mainly been investigated by means of oral form recognition (2;3). In these studies, participants were asked to match the shape of the objects presented intraorally with shapes presented visually. Williams and La Pointe (4) also included interdental size and weight discrimination tests; they observed that participants able to detect small weight differences inter-dentally were good at recognising shapes. Anstis and Loizos (5) compared visual, digital and intra-oral presentation on the size judgements of small holes; they found that holes were judged larger when presented to the tongue or eyes than when presented to the finger. La Pointe *et al.* (6) asked their participants to make size judgements matching intraorally presented holes with reference arrays presented visually and digitally; they demonstrated that individuals tend to over-estimate the size of holes placed in the mouth. Lamey *et al.* (7) and Bittern and Orchardson (8) also found mismatching in the direction of over-estimation when individuals were asked to match the size of a hole intra-orally with holes assessed with the fingers; he latter showed that the depth of the hole was not of importance, whereas the shape was; peg stimulation did not result in over-estimation. Melvin and Orchardson (9) found that the mismatch is due to the inability of the fingertip to access small comparator holes. Also, Anstis (10) tested their participants in a task comparing the apparent size of holes felt with the tongue. The largest effect of over-estimation was reported for the smaller holes in all the above studies, except for La Pointe *et al.* (6), where the over-estimation was greatest for large holes. This discrepancy could be due to differences in the form of the intraoral objects.

Speirs *et al.* (11) investigated the ability to assess liquid volumes. They found that the error in perception was considerable and it was greater for smaller volumes, where there was a tendency to over-estimate.

The above-mentioned studies have focused mainly on the size perception of holes. Little published information is available on the intraoral size perception of objects. Dellow *et al.* (12)

investigated the oral assessment of plastic cylinder size and found that their participants made errors of over-estimation.

The present study combines oral and visual size assessment of objects, as Anstis and Loizos (5) and La Pointe *et al.* (6) noted that incorrect judgements of the size of holes were greater for digital matching than for visual.

We were interested in how the size of an object is perceived in the mouth and specifically in the role of the palate and the tip of the tongue, as these are the most sensitive regions of the mouth (13). A secondary goal was to investigate the relationship between volume and weight in the determination of the size of an object.

MATERIALS & METHODS

Ten healthy individuals (two males, eight females, average age 36 years) without neurological or other disorders participated in the study. Before the experiment, they were informed about the procedures.

The chosen stimuli were ball bearings (Dejay, Wokingham, UK) in eight sizes varying from 2 to 15 mm diameter (Table 1) and in four different materials with different densities: steel, nylon, PTFE, and polypropylene (8.0, 2.2, 1.1, and 0.9 g/cm³, respectively). Not all sizes were included in the set administered to the participants; balls 0, 6 and 7 were only included in the reference set and were not administered, which allowed individuals to under-estimate the smallest object presented to them and to over-estimate the largest; they were not informed of this restriction. Balls of four different materials in five sizes were presented four times at room temperature to the subjects, resulting in the use of a total of 80 ball bearings this study.

Table 1. Diameter and weight of the spheres and materials included in the experiment

Material	7	6	5	4	3	2	1	0
	Ø (mm)							
	15.0	11.1	9.53	7.94	6.35	4.76	3.97	2.0
Weight (g)								
Steel			3.60	2.10	1.06	0.45	0.26	
PTFE			0.51	0.30	0.15	0.07		
Nylon			1.01	0.56	0.29	0.12	0.07	
Poly P			0.39	0.23	0.12	0.05		

Participants were seated comfortably and in an upright position at a table opposite the researcher. The balls were randomly administered in black cups. In front of the participants a reference set of eight numbered steel balls (0-7) of increasing size was displayed, together with collection cups numbered from 0 to 7 (Fig 1). The participants were instructed to pour the ball

from the cup into the mouth to eliminate any visual or tactile cues about the object's size. Then, with the ball still in the mouth, the participant compared its size with the size of the balls in the reference set. The ball was then spat out into the collection cup that had a number corresponding to the size of the ball in the reference set. Participants received no feedback on their performance during or after the experiments, to ensure that they could not improve with practice.



Fig 1. The experimental set-up from the subject's view, including reference set, collection cups, administration cups and the set of spheres yet to be administered.

To investigate the influence of the palate on the volume perception and assessment the experiment was then repeated with participants wearing a custom-made, 1mm thick, vacuum-formed plastic palate, prepared from dental impressions taken during a previous session, that covered the hard palate and occlusal surface of the upper teeth. Participants were allowed to get used to the plastic palate for some time before proceeding with the experiments.

Performance was quantified, thus, correct-estimations were scored 0; under-estimations, the ball reported as smaller than it actually was, scored -1; and over-estimations, where the ball was reported as larger than it actually was, were given the score +1. At the end of the experiment, the number of under-, over-, and correct-estimations was counted for each size and for each material. In addition, the mean response was calculated for the administered ball sizes.

Statistics

Principal-component analysis (PCA) was used to create and compare clusters. The statistical significance of differences between conditions was calculated by one-way ANOVA, with $p < 0.05$ considered significant.

RESULTS

No significant differences in size perception were found between the different materials, suggesting that density plays no part in the intraoral perception of size. Accordingly, data acquired with all four materials were pooled for analysis. However, when interviewed, subjects did report a noticeable difference in mouth feel (temperature and surface structure) between the steel and the plastic balls, but were unable to discriminate between the different plastics.

The mean responses for the different ball sizes are shown in Fig 2. These data show that there was a tendency (for ball size 1, $0.05 < p < 0.1$) to under-estimate the size of ball bearings up to about 8 mm diameter (ball size 4), where the number of over- and under-estimations was similar. The plastic palate had the effect of improving performance and specifically of decreasing the percentage under-estimations (Table 2).

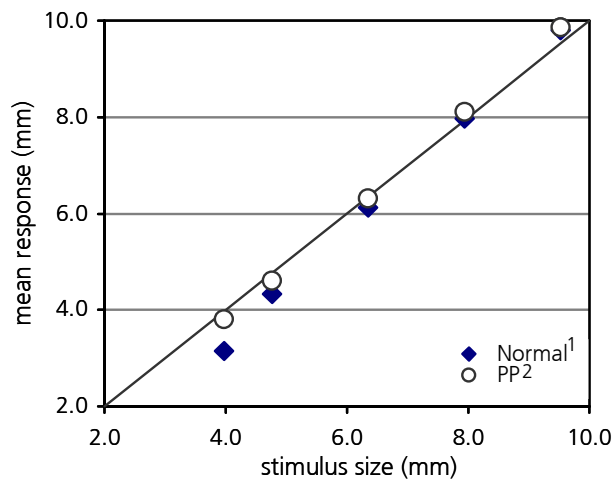


Fig 2. Showing subjects' mean scores for size estimation vs. object diameter, with and without full palatal coverage. The full line represents total agreement between stimulus size and response.

¹Normal conditions. ²With custom-made plastic palate

When participants were wearing the plastic palate, they perceived the balls to be larger than without it; this difference was significant ($p < 0.05$). The point at which the over- and under-estimations reach similar values occurred at a smaller diameter when wearing the plastic palate than during normal conditions, 6.5 mm (ball size 3) versus 8 mm (ball size 4).

Table 2. The percentage under-, correct and over-estimations, and means response of ball size, with and without palatal coverage.

	Ball number and size									
	1 (3.97 mm)		2 (4.76 mm)		3 (6.35 mm)		4 (7.94 mm)		5 (9.35 mm)	
	Norm ^a	PP ^b	Norm ^a	PP ^b	Norm ^a	PP ^b	Norm ^a	PP ^b	Norm ^a	PP ^b
Under-estimated (%)	46	26	47	35	35	24	22	14	18	12
Correct-estimated (%)	42	54	45	51	46	54	52	63	47	57
Over-estimated (%)	11	19	8	14	19	22	26	23	34	31
Mean response (mm) (S.D.)	3.1 (0.6)	3.8 (0.9)	4.3 (0.9)	4.6 (1.0)	6.1 (1.2)	6.3 (1.4)	8.0 (1.7)	8.1 (2.1)	9.8 (1.9)	9.8 (2.2)

DISCUSSION

Density is known to be sensed orally, at least unconsciously, altering the processes of oral transition and swallowing (14), which might therefore, suggest that density would influence size perception, with a heavier ball being perceived as larger; this was not the case, despite the nine-fold difference between the least and most dense materials. Several other sensory cues, such as surface finish and thermal conductance, also allowed the material to be distinguished. The steel balls were reported to feel cold and the plastic ones warmer, but participants were able to compensate for these differences without it influencing the size perception, suggesting that oral assessment of size is based simply on the dimensions of the object and not on its weight or other characteristics.

Anstis and Loizos (5) investigated the extent of cross-modal illusion in the assessment of hole size by comparing all combinations of eye, tongue and finger presentation and matching; they report size illusions for tongue-finger and eye-finger, but less for tongue-eye matching tasks. Others have investigated intraoral size perception by asking their participants to compare the size of holes felt with the tongue with a manually presented reference set (7;15). La Pointe *et al.* (6) used a reference set by touch only as well as a visual one. Speirs *et al.* (11) used a visual assessment of liquid volume. These studies have shown a consistent over-estimation of size, especially when object diameter was less than 10 mm. Interestingly, La Pointe *et al.* (6) showed that visual comparison resulted in less over-estimation than did the manual method (58 % versus 87%); they found that the disparity of matching was greatest with large holes.

In contrast, in our study, participants under-estimated the size of the balls. Under normal conditions, the under-estimation averaged 33.6% overall. When wearing the custom-made plastic palate, their under-estimation was significantly lower ($p < 0.05$) and reduced to an average of 20.8%. The differences between previous findings and those obtained here are explained by the difference in experimental design. When assessing a hole in a plastic cube or



plate, only the tip of the tongue is used for sensory input. The tongue can sense the full extent of the holes, including the edges, and subjects tend to over-estimate size. A ball on the other hand, is spherical and the area of contact with the palate is small in comparison to the diameter. However, when assessing the size of a spherical object, the tongue presses it against the palate, and sensory input from both is obtained. The tongue wraps around the sphere to sense size, but this input is overridden to an extent by input from the hard palate, which can only sense the rather small area of contact. When a plastic palate is worn, the input from the individual's palatal mucosa is masked and size is estimated solely on basis of input from the tongue.

Grasso and Catalanatto (2) and Oliver (16) found no significant differences in oral form recognition between participants with and without full palatal coverage. Garrett *et al.* (17) reported no significant differences in oral stereognostic ability in denture wearers with and without their dentures in place; they concluded that the teeth and the receptors in the periodontal ligament are of minimal importance in the oral detection of shape. However, during the recognition of forms, exact size is of minor importance and the input from the tip of the tongue is sufficient without input from the palate to recognise shape. One could wonder why the palatal coverage slightly improved our participants' size assessment, when impaired masticatory performance is reported for full-denture wearers. This finding supports the idea that impaired chewing in denture wearers is not due to decreased size information as a result of coverage of the palate, but to decreased muscle function and instability of the protheses.

The percentage correct answers with, as well as without, the plastic palate were considerably higher (mean 50%, 56% respectively) than they would have been due to chance alone (12.5%, one out of eight references). It is remarkable that while the most subjects scored the balls randomly around the correct answer, one consistently over-estimated and another was consistent in underestimating the balls' sizes throughout the experiment.

Considering these two who were consistent in their over- and under-estimations, one might wonder whether this would also influence their perception of food breakdown and the size of the fragmented particles. Would the overestimating individual chew the food into smaller particles before deciding to swallow than the underestimating subject, who might swallow larger pieces?

This study demonstrates that oral size perception results from a combination of sensory inputs from the palate and the tongue. The use of a plastic palate to minimise the input from the palate resulted in less under-estimation. The object's weight had no effect on size assessment. We found an under-estimation, while Dellow *et al.* (12) describe an over-estimation of cylinder size in the mouth; this difference could well be attributable to the difference in stimulus shape, with the cylinders differing in two dimensions. Further work is required to understand the mechanisms of oral size perception and its role in the control of bite size and swallowing.

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CHAPTER 3

RELATIONSHIP BETWEEN ORAL SENSITIVITY AND
MASTICATORY PERFORMANCE

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Journal of Dental Research in press



In press

ABSTRACT

The size of a bolus determines how it will be manipulated in the mouth and swallowed. We hypothesized that mucosal sensitivity would be important for masticatory function. The accuracy of solid object size perception, spatial acuity and food particle size reduction during mastication were measured in 22 healthy adults with/without topical anesthesia of their oral mucosa. Topical anesthesia had no effect on the perception of sphere sizes, but significantly reduced spatial sensitivity. Without anesthesia, there was a correlation between an individual's ability to perceive the size of steel spheres (4-9 mm diameter) and the sizes of food particles chewed for 15 cycles and at swallowing. There was no correlation between spatial sensitivity and food particle size. We suggest that the stimuli used to test two-point discrimination only stimulates superficial receptors, which involve light touch and are easily anesthetized, while the spheres might excite more deeply-set receptors. The latter appear more important for masticatory performance and swallowing.

INTRODUCTION

Sensitivity of the mouth includes the ability to assess shape, size, and surface texture. Oral sensitivity has often been measured to track damage and rehabilitation after strokes (1), prosthodontic treatment (2;3), and for speech disorders (4). Various methods to measure oral sensitivity have been employed, including oral form recognition (2;5-8), interdental size and weight discrimination tests (9), intra-oral size judgments of small holes (10-13), cylinders (14), spheres (15), and two-point discrimination (16). The last named method has been standard since the 1860s and remains the most commonly used to determine a subject's tactile spatial resolution.

Information on the significance of various oral components in oral size perception and sensitivity is required to understand their role in controlling mastication and swallowing. In the present study, we were specifically interested in oral sensitivity to size. Size can be sensed by pressure and stretch receptors in tongue and palate mucosa, in addition to mechanoreceptors in the periodontal ligament (17). Oral perception of size does not depend on the density or material of an object, but solely on its actual size and shape and results from a combination of sensory input from the tongue and palate (15). In the present study, we excluded input from the superficial layers of mucosa by applying topical anesthesia to the tongue and palate.

Masticatory function has been studied in various groups of subjects, such as dentate subjects (18), partial and complete denture wearers (19), and subjects with implant-retained overdentures (20). Masticatory performance is significantly reduced when dentures replace natural teeth. The most common way to study masticatory function is to determine an individual's capacity to grind or pulverize a test food by analyzing the chewed material (21). To assure safe swallowing, the particle sizes in the bolus of the chewed food need to be detected. It has been suggested that the main site for detecting food particles is not between the teeth, but on oral mucosa (22). In addition, tongue motor skill is significantly correlated with masticatory performance (23). Thus, information from oral mucosa, *e.g.* oral sensitivity, may be related to measurements of masticatory performance. If median swallowing particle size is related to the ability to assess objective size, the question arises whether a subject with good discriminative abilities also chooses to swallow smaller particles.

The aim of the present study was three-fold: Firstly, we were interested in how size is perceived in the mouth and whether the ability to assess size is related to the spatial sensitivity. Secondly, we studied how these features are influenced by topical anesthesia. Finally, we wanted to study how the median particle size at swallowing and masticatory performance are related to the ability to assess size of objects and sensitivity in the mouth.

We hypothesized that topical anesthesia would affect oral perception of size and spatial acuity, and that the particle size at swallowing would depend on oral mucosal sensitivity.



MATERIALS & METHODS

Twenty-two healthy individuals (13 female and 9 male, with an average age of 27.6 years) participated in the study on two-point discrimination and masticatory performance. Fourteen of these subjects (8 female and 6 male) also participated in the study on oral size perception. The Ethics Committee of the University Medical Center approved the protocol. Written informed consent was obtained from each subject after a full explanation of the procedure. All treatments and stimuli were administered in random order within each part of the study.

Oral perception of object size

The chosen stimuli for oral perception of size were steel spheres (Dejay, Wokingham, UK) in five sizes, varying from 4 to 9 mm. The spheres were given to the subjects, one at the time, in black cups. A reference set of eight spheres numbered zero to seven was displayed in front of the subjects. The reference set included the five test sphere sizes plus 2, 12, and 15 mm spheres. The extra sphere sizes allowed subjects to under- or overestimate perceived sizes. The subjects were not informed about the extra sizes in the reference set. The subjects were instructed to transfer the sphere from the cup directly into the mouth without any visual or tactile cues about the object's size and to assess the size of the sphere between the tongue and palate. Then, with the sphere still in the mouth, the subject matched its size with the size of the spheres in the reference. The sphere was then spat into a collection cup with the number corresponding to the size of the sphere in the reference set. The procedure was performed according to (15). The same procedure was then repeated after administering topical anesthesia. Topical anesthesia was applied by spraying liquid lidocaine (Xylocaine 10 mg, AstraZeneca, Zoetermeer, Netherlands) on a cotton wool roll and rubbing the roll over the tongue or tongue and palate. The anesthetic was left for two minutes, after which the subjects rinsed out any surplus with water.

Two-point discrimination threshold

The minimum separation of two punctiform stimuli that can be discriminated as two distinct points was determined by lightly pressing two pins onto the anterior part the tongue, with and without local anesthesia. The separation of the pins ranged from 0-8 mm, and the staircase method was used, with steps of 1 mm. The subjects were instructed to indicate whether they felt one or two stimulus points. Topical anesthesia was applied as described above.

Masticatory performance and swallowing threshold

In an initial test we determined the masticatory performance of the subjects by quantifying the degree of fragmentation of an artificial test food, Optocal Plus (20) without local anesthesia. The subjects chewed on portions of 17 cubic particles (edge size 5.6 mm, totaling approximately 3 cm³) for 15 chewing strokes. The degree of fragmentation of the chewed food was determined by sieving the food through a stack of 9 sieves with apertures decreasing from 5.6 to 0.7 mm and a bottom plate. The amount of test food on each sieve and on the bottom plate was weighed. The distribution of particle sizes of the comminuted test food can be

mathematically described by a cumulative function (24) with the degree of fragmentation of the food given by the median particle size, X_{50} , which is the aperture of a theoretical sieve through which 50 percent of the weight of the comminuted food could pass. In a second test, the subjects were instructed to chew until they were ready to swallow, but instead of swallowing, they spat out the test food. We determined the number of chewing strokes until the urge to swallow and determined the degree of fragmentation of the chewed food at the swallowing threshold.

Data analysis

Object size perception was compared to the standard line, and the effect of the different treatments (control, anesthetized tongue and anesthetized tongue and palate) was analyzed with regression analysis. Paired t-test was performed to analyze the difference between actual and perceived object size in the mouth and to analyze the effect of anesthesia on two-point discrimination performance. Pearson's correlation coefficients were calculated for the relations among object size perception, two-point discrimination, and the different parameters of masticatory performance. All analyses were performed with SPSS (9.0 SP 4M, SPSS inc., Chicago, IL). $P < 0.05$ was considered significant.

RESULTS

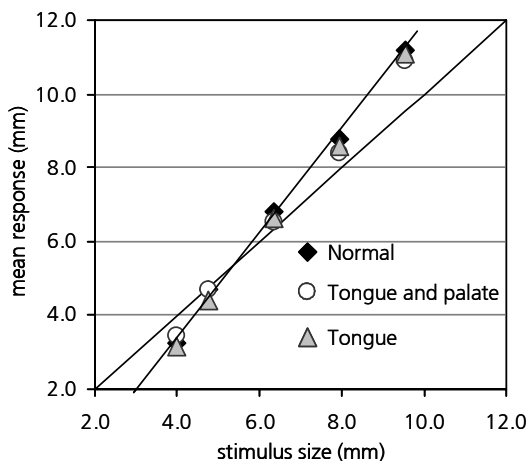


Fig 1. The relation between actual and perceived sphere size during normal condition, with anesthetized tongue and palate, and anesthetized tongue. The thin line through the origin depicts the standard line where the perceived size equals the actual particle size.

Regression lines of perceived object size after different treatments were determined (Fig. 1). Anesthetizing either the tongue or the tongue and palate had no significant effect on the subject's ability to assess size, and accordingly, the subsequent analysis included the mean of the two treatments and the untreated condition. The slope of the regression line for the perceived object size (1.37 ± 0.02 , $N = 280$) was significantly steeper than 1.0 ($p < 0.001$). A comparison between the actual and subjective object size revealed that there were significant differences at the far ends of the line, where small spheres (4.0 mm) were underestimated, while the size of spheres of 9.4 mm were overestimated.

Means and standard deviations for the two-point discrimination test, median particle size (X_{50}) after 15 chewing strokes (15x) and at swallowing, number of chewing strokes and chewing time until swallowing are shown in Table 1. Topical anesthesia had a significant effect on the tactile spatial resolution of the tip of the tongue, where the threshold increased on average with $0.4 \text{ mm} \pm 0.7$ ($N = 22$) as observed in the two-point discrimination test. The median particle size (X_{50}) at swallowing varied between 1.0 - 2.5 mm ($N = 22$, mean $1.7 \text{ mm} \pm 0.4$), and was related to the median particle size after 15 chewing cycles ($N = 22$, range: 1.6 - 4.9, mean $3.5 \text{ mm} \pm 0.8$). The number of chewing cycles and the time until swallowing were strongly and positively correlated ($N = 22$, $r = 0.88$; $p < 0.001$).

Table 1. Means and standard deviations for the two-point discrimination test, median particle size (X_{50}) after 15 chewing strokes (15x) and at swallowing, number of chewing strokes (N) and chewing time (I) until swallowing.

	Mean	St. Dev.	N
$X_{50}(15)^a$ (mm)	3.5	0.8	22
$X_{50}(\text{swallow})^b$ (mm)	1.7	0.4	22
N_{swallow}^c	36.0	12.2	22
T_{swallow}^d (s)	26.4	8.9	22
2-point normal ^e (mm)	2.4	0.9	22
2-point anesthesia ^f (mm)	2.8	1.2	22

a Median particle size after 15 chewing cycles

b Median particle size at swallowing

c Number of chewing cycles needed to prepare for swallowing

d Time needed to prepare for swallowing

e Two-point discrimination threshold on untreated tongue (mm)

f Two-point discrimination threshold on topically anesthetized tongue (mm)

The correlation coefficients of perceived object sizes, masticatory performance, and two-point discrimination thresholds are depicted in Table 2. The median particle size after 15 chewing strokes was positively correlated with the oral perception of object size for particles of 6.4 mm and larger. However, no such relation was observed for median particle size at swallowing (X_{50} (swallow)). There was no relation between the spatial resolution on the tongue and the oral ability to perceive object size, nor with the median particle sizes, chewing time, and number of strokes.

Table 2. A matrix showing the correlation coefficients among perceived particle size of spheres of five different sizes, median particle size (X_{50}) after 15 chewing strokes (15x) and at swallowing, number of chewing strokes (N) and chewing time (T) until swallowing, and the two-point discrimination test.

	4 mm	4.8 mm	6.4 mm	7.9 mm	9.4 mm	X_{50} (15x)	X_{50} swallow	N swallow	T swallow	2-p normal
Sphere size 4.8 mm	0.82**									
Sphere size 6.4 mm	0.57*	0.87**								
Sphere size 7.9 mm	0.58*	0.80**	0.94**							
Sphere size 9.4 mm	0.56*	0.78**	0.89**	0.94**						
X_{50} (15x) a	0.06	0.24	0.58*	0.57*	0.53*					
X_{50} swallow b	0.22	0.19	0.37	0.45	0.25	0.49*				
N swallow c	-0.04	-0.07	-0.22	-0.37	-0.26	0.21	-0.51*			
T swallow d	-0.23	-0.06	-0.15	-0.37	-0.18	0.24	-0.51*	0.88**		
2-p normal e	-0.03	-0.13	-0.27	-0.13	-0.04	-0.10	-0.11	-0.03	0.13	
2-p anaesthesia f	0.07	0.06	-0.15	-0.07	-0.02	-0.24	-0.34	0.16	0.23	0.82**

N = 14

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

a Median particle size after 15 chewing cycles (mm)

b Median particle size at swallowing (mm)

c Number of chewing cycles needed to prepare for swallowing

d Time needed to prepare for swallowing (s)

e Two-point discrimination threshold on untreated tongue (mm)

f Two-point discrimination threshold on topically anesthetized tongue (mm)



DISCUSSION

When the objects in the mouth were matched with a visual reference set, the size of small spheres was underestimated, medium spheres correctly estimated, and large spheres overestimated. These results are in conflict with previous studies (10) that found no such disparity when visually matching the size of holes in objects to intra-oral determinations. The discordance between these studies can be explained by the difference in experimental design (15). When assessing a hole, the tip of the tongue can sense the full extent of the hole, including the edges. During the assessment of sphere size, the sphere would be manipulated in the mouth by pressing it against the palate by the tongue. The area of the sphere touching the palate would be much smaller than the actual diameter. The tongue senses the whole diameter, while the palate only senses a small part of it, resulting in conflicting information, with spheres being perceived in the mouth as smaller than they actually are. Covering the palate with an acrylic plate removes this effect.

The observation in the present study that topical anesthesia had no effect on size perception was surprising. An explanation for this could be found in the degree to which the mucosa was anesthetized. The result of two-point discrimination, where the anesthetized tongue was significantly less sensitive, shows that the anesthesia was effective. This is in line with other studies (25) where superficially anesthetized subjects needed significantly longer time to complete an oral discrimination task. We suggest that the two-point discrimination test only stimulates superficial receptors, which involve light touch and are easily anesthetized. During the manipulation of the spheres, however, the spheres could be pressed against the palate and excite sensors set more deeply in the tongue. In this way, the effect of topical anesthesia might exclude superficial receptors, but possibly still include deeper-situated receptors.

A positive correlation between the median particle size at swallowing and after 15 chewing was observed. This is consistent with previous studies (26-28) and suggests that good chewers (small median particle size) often swallow boluses containing smaller particles. In accordance with a previous study (27), no correlation was found between masticatory performance and the number of chewing cycles to prepare food for swallowing. It follows that, poor chewers do not compensate for their reduced chewing performance by using more chewing strokes. In this study, we observed a negative correlation between the number of chewing strokes until swallowing and median particle size at swallowing. This is consistent with results from an unpublished study (Van der Bilt *et al.*), performed on 80 subjects and it does seem logical that a larger number of chewing strokes results in smaller particles. The time until swallowing shows similar results to those found for the number of chewing cycles, due to the high correlation between these variables.

We found a positive correlation between size perception and median particle size after 15 chewing strokes. Poor chewers estimated the spheres to be larger than the good chewers and more often overestimated the sphere size. This implies that poor chewers swallow larger particles because their chewing ability, not their oral sensitivity, is reduced. It would appear

that they are or have become even more sensitive to sizes of particles that would cause discomfort during swallowing. This suggests that poor chewers are more cautious about large food particles, and possibly that they would initiate deglutition even with a bolus containing particles up to 5 mm. Prinz and Lucas (1995) (22) suggest that the upper size limit of particles that are swallowed is dictated by the individual's tolerance of discomfort from distension of soft tissue in pharynx and esophagus. Good chewers can easily comminute food to particles well below this upper tolerance level and as a result they are less sensitive to the larger object sizes.



The fact that no correlation was found between spatial acuity and assessment of sphere size indicates that the two measurements are unrelated. Spatial acuity only describes a lower limit of sensitivity, which does not overlap with the size of the spheres used in this study. Hence the ability to assess object size in the mouth cannot be predicted by the spatial acuity of the tongue.

In conclusion, oral perception of the size of small spheres is underestimated, and the size of large spheres is overestimated. Topical anesthesia reduces spatial acuity, but does not affect the perception of sphere size. We suggest that two-point discrimination only stimulates superficial receptors, which involve light touch and are easily anesthetized, while the spheres might excite more deeply-set receptors. These receptors appear critical to masticatory performance and swallowing. Poor chewers are sensitive to sphere sizes that could cause them discomfort while swallowing. These results invite more research on oral sensitivity in healthy and orally impaired subjects in order to gain more insight into the mechanisms controlling chewing, swallowing, and object perception.

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CHAPTER 4

ORAL SIZE PERCEPTION OF PARTICLES: EFFECT OF SIZE, TYPE, VISCOSITY AND METHOD

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In preparation

ABSTRACT

Three studies were performed to investigate how different parameters affect oral size perception of small particles. SiO₂ and polystyrene of sizes varying between 2 and 230 μm were included in the study. The particles were mixed into a custard-type medium of two viscosities, thickened with carboxy methyl cellulose. 18 healthy subjects assessed the coarseness of particles by rubbing the sample between the tongue and palate. In the first study the importance of size and type of particle, and viscosity of the dispersion medium were studied by direct-scaling. The stimuli were rated in comparison to anchor stimuli. The second study addressed the relative importance of tongue and palate in oral size perception by applying topical anaesthesia and the coarseness was again assessed by direct-scaling. In the third study the effect of size and type of particle on size perception was also studied by forced choice ranking, where after the results of the two methods – direct scaling and forced ranking were compared. The results show that the size and characteristics of the particle were of importance for perception of particle size, where hard and irregular particles were perceived as larger than soft and round of similar size. The viscosity of the dispersion medium had no significant effect. Topical anaesthesia of tongue and palate did not produce results different from the control situation. Finally, the two methods of size perception, direct scaling and forced ranking produce very similar results on oral size perception.

INTRODUCTION

Oral sensitivity to particles has previously been investigated by using objects of different shapes (1-8), sizes and materials (9;10). Texture, e.g. grittiness of microcrystalline cellulose and pulverized food particles in the mouth have also been examined (11-13). For oral perception of grittiness, it has been reported that concentration, size and shape of the particle are of importance, as well as the medium in which they are dispersed (11;13;14). Grittiness perception increased with increasing particle size, concentration and sharpness. Conversely, grittiness decreased in more viscous media. Assuming that grittiness depends on how well the particles are sensed, we assume that these factors are important also for oral perception of size.

It would be interesting to know where in the mouth the size of small particles is perceived. One approach to this is to study the relative importance of the tongue and palate in size perception. Previous research in this laboratory (9) has shown that when assessing the size of spheres, ranging from 4 -9 mm, the sizes of the smaller spheres were underestimated. However, this illusionary effect was reduced when using a plastic palate to minimize the palatal input. Conversely, the application of topical anaesthesia to the tongue and/or palate had no effect on the perception of size (10). This could be attributed to the degree of anaesthesia, where only the superficial layers were affected. The spheres were pressed against the palate forming an indent and it is suggested that the pressure exerted was sufficient to fire receptors in deeper layers of the epithelium. In the case of small particles, we hypothesize that the pressure exerted on a single particle is smaller and hence more superficial receptors could be involved.

Tongue and palate are expected to react differently to the same stimulus as they have different densities of slowly and rapidly adapting afferents (15). A majority of the afferents of buccal mucosa, facial skin and lip is slowly adapting, with small and well-defined receptive fields(16;17). On the contrary, the majority of the superficial afferents of the tongue are rapidly adapting, with extremely small and well defined receptive fields (18). Slowly adapting receptors continue to discharge, while rapidly adapting receptors cease to discharge during maintained tissue deformation. The extraordinarily high acuity of the tip of the tongue for form and texture is associated with a prevalence of rapidly adapting afferent (15).

There are numerous ways of reporting and quantifying sensory sensations. When assessing a fixed dimension such as size, a quantitative scale is preferred. However, when comparing sizes, deciding which is larger, a standard has to be set, in order to keep the scale relevant. Even a difference in diameter of 1000 x (e.g. 1 μm vs. 1 mm) might disappear, if a subject decided on comparing all the particle sizes with e.g. a football. Thus by giving the end-points of the scale in the form of anchor stimuli, that problem is circumferenced. The anchors are however never administered simultaneously with the stimuli. As a result, a correct comparison of the particle sizes relies on a good memory.

Another way to look at the ability to rate size is to rank the samples. By doing this, the stimuli are directly compared with each other, so the ability to rank the samples is a direct measure of



oral sensitivity. A drawback of this method is that it is not absolutely quantitative, but gives only a relative measure. In addition, only the samples included in the same set can be compared.

Since both methods have their pros and cons, we wanted to use both and compare the outcomes, to see to what extent the results of the methods give similar results as to the subjects' oral perception of size.

The aim of the present study was three-fold: Firstly, to investigate the importance of type and size of particles and of the viscosity of the dispersion medium on oral size perception of particles. Secondly, we sought to study how size of particles is perceived in the mouth and what the relative influence of tongue and palate is on the size perception. Thirdly, we wanted to compare two methods of assessing size: direct scaling in comparison to anchor stimuli and relative comparisons of forced rankings.

MATERIAL & METHODS

Subjects

Eighteen (13 female and 5 male) trained adult panellists participated in the study. Their age ranged between 20 and 36, average age was 23 years. The subjects were selected on the basis of a well functioning smell and taste perception. They gave informed consent and were compensated for their participation. Each subject was always tested at the same time of the day. All 18 subjects participated in the study on the influence of tongue and palate in size perception and 12 of these participated in the direct-scaling and forced ranking studies.

Stimuli

Dispersion medium

Dispersion medium was prepared in two viscosities (3 and 6 Pas) in the laboratory by blending 8.5 g, or 11.25 g Carboxy Methyl Cellulose (Akucell AF3295 Akzo Nobel, Amersfoort, the Netherlands), 62.5 g sugar and 1.5 g vanilla flavour (3912 Danisco) and thereafter add the dry blend to 1 litre of commercial full-fat milk (3 %) during mixing. The product was mixed in a professional mixer for 25 minutes. 5 minutes before the end, 1 ml of yellow food colorant (Egg yellow, Supercook, Leeds, UK) was added per litre of product to enable the colour to mix in thoroughly. The products were prepared on the day of evaluation and stored and administered at 10°C.

Particles

Silica dioxide (SiO₂) (2.5 g/cm³; U.S. Silica company, Ottawa, IL) (Fig 1a) in different size grades was ordered from the manufacturer. These grades were sieved into discrete classes; 20-50 µm, 50-100 µm, 100-150 µm, and 150-200 µm (Interlab B.V., Etten-Leur, Holland). The median particle sizes of these classes were determined by Coulter laser diffraction to be 40, 80, 135 and 180 µm, respectively. In addition, the grade "Min-u-sil 5", had a median particle size

of 2 μm . Spherical polystyrene particles (1.10g/cm³; Dynoseeds®, Polymer-systems.com) (Fig 1b) were ordered from the manufacturer in the discrete sizes 40, 80, 140, and 230 μm . In table 1, the sizes and types of particles included in the study are given. Imai et al (13) have shown that the concentration of particles is of importance for the perception of grittiness. It can be hypothesized that the number of particles is crucial, as opposed to the weight of the particles. Therefore we chose to add approximately the same number of particles of both types of particles to the dispersion medium. Since the density of SiO₂ is more than twice as high as of the polystyrene particles, we added 5% weight of SiO₂ and 2.3 % weight of polystyrene particles to the custard dessert to assure similar numbers of particles. The particles were mixed well with the dispersion medium and the stimuli were placed in 25 ml containers prior to sensory evaluation.

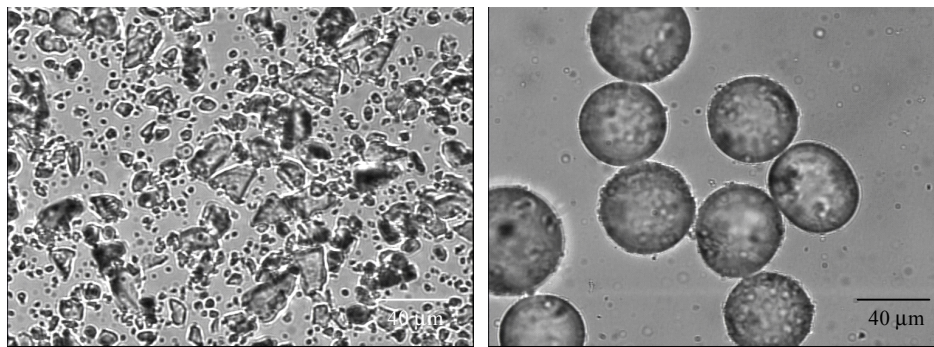


Fig 1. Micrographs of a. silica dioxide and b. polystyrene samples with a mean diameter of 40 μm

Table 1. Sizes of the two types of particles (SiO₂ and polystyrene) included in the study.

Mean Size (μm)	0	2	40	80	140	230
SiO ₂		X	X	X	X	
Polystyrene	Control		X	X	X	X

Size perception

Direct scaling – the effect of size and type of particles, and of medium viscosity

Subjects were instructed to place the stimuli on the tongue and rub the tongue against the palate in order to identify particles. Thereafter the sample was swallowed and the mouth thoroughly rinsed with water. The subjects received two anchor stimuli: one containing no particles, the subjects were informed that these were the smallest particles they would receive and these were to be rated at the beginning of the line scale; the other anchor stimulus containing SiO₂ particles of 250 μm , the instruction was that these were the largest particles

included in the study and were to be rated at the far end of the scale. The anchors were redistributed four times during the experiment in order to recalibrate and refresh the subjects' memory. Duplicates of all combinations of particle size and type, and medium viscosity were administered to the subjects at random during a two-hour session. The assessment took place on a one to one basis, with the sole objective of rating the size of the particles. Subjects' responses were scored on a 100-point VAS response scale, ranging from fine to coarse.

Influence of tongue and palate in size perception

Topical anaesthesia was applied by spraying liquid lidocaine (Xylocaine 10 mg, AstraZeneca, Zoetermeer, Netherlands) on a cotton wool roll and rubbing the roll over the tongue or palate. The anaesthesia was left for two minutes, after which the subjects rinsed out any surplus with water. This regime has been used previously (Engelen et al. submitted) and was shown to be potent enough to significantly reduce the spatial acuity of the tongue, as measured by two-point discrimination. Three sizes of SiO₂ (median 40, 80 and 135 µm) were mixed into a medium with the viscosity of 3 Pas. The experimental protocol was identical to and the subjects were asked to handle the product in the same way as described in the direct scaling section above. The size of the particles were rated in comparison to anchor stimuli and rated on a 100-point VAS scale, ranging from fine to coarse. The two anaesthetics-regimes (tongue and palate) were compared with the control situation, where no anaesthesia had been applied.

Forced ranking

Samples were placed in random order in front of the subjects. The subjects were instructed to rank the stimuli by forced choice from fine to coarse. As in the regime for rating described above, they were told only to sample the stimuli between the tongue and palate. The subjects received no restraints considering the time, nor the amount of comparisons between the samples they were allowed to make. All sizes and both types of particles were included in duplicates. Hence, the study with SiO₂ consisted of six samples (five different sizes, plus a control without particles), whereas the polystyrene study consisted of five samples (four sizes, plus control). In this study only the 3 Pas medium was included, which limited the number of samples, in order to prevent fatigue. Following the order in which the products were ranked, they were numbered 1 to 6 (SiO₂), or 1 to 5 (polystyrene), indicating fine to coarse.

Data Analysis

Direct-scaling

Repeated-measures ANOVAs were performed with Greenhouse-Geisser as correction factor on data averaged across duplicates (SPSS 9.0 SP 4M, SPSS inc., Chicago, IL). Size and type of particle and viscosity were included as within-subject factors.

Influence of tongue and palate in size perception

The same analysis was performed to investigate the effect of particles and topical anaesthesia on rated particle size. Size of particles and anaesthesia regime were included as within-subject factors.

Forced ranking

To determine the degree of successful ranking, each subject obtained a score through an elaborate scoring system taking the relative order of the rankings into account, with the score for each individual defined as

$$\sum_{i=1}^{n-1} \sum_{j=i+1}^n I(x_i < x_j) \quad \text{where } I(x_i < x_j) = 1 \text{ if } x_i < x_j \text{ and } 0 \text{ if } x_i > x_j \text{ and } x_i \text{ equals the observed}$$

ranking. The highest possible score was 15 (SiO₂) or 10 (polystyrene) and the lowest 0. In this manner an absolute measure was acquired as to the subject's ability to rank the size of the particles. The scores are approximately normal distributed with mean 7.5 and standard deviation 2.7 for SiO₂, and mean 5.0 and standard deviation 2.0 for polystyrene when assuming random ordering of the samples. To test whether the rankings were performed at random, or whether the subjects used the actual size to order the samples from small to large, a z-test was performed. P < 0.05 was considered significant.

Comparison of two methods of size assessment

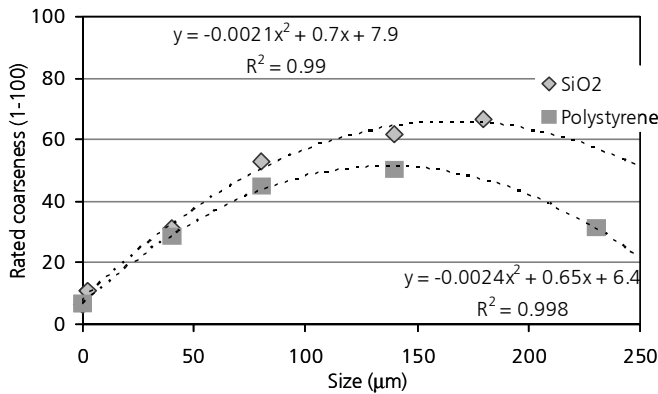
The ratings from the direct scaling were converted into rankings and scores were calculated for each subject according to the method described above. To compare the two types of method (direct scaling and rankings), the obtained scores per person were compared in a paired t-test. P < 0.05 was considered significant.



RESULTS

Direct scaling – the effect of size and type of particles, and medium viscosity

Figure 2 illustrates the effects of size and type of particle on size perception of particles with the direct scaling method. In table 2, the mean and standard deviation of the ratings for the different sizes, two types (SiO₂ and polystyrene) and two viscosities (3 and 6 Pas) are shown. ANOVA reveals that there were significant main effects of size ($p < 0.001$) and type ($p = 0.01$) of the particles on the way the size was perceived. Subjects were well able to rate the size of particles, and the response increased with increasing particle size up to 140 μm , where the functions seem to have an optimum or level off. Fitting a second degree polynomial on the data resulted in an R^2 of 0.99 for SiO₂ and 1.0 for polystyrene (Figure 2). SiO₂ was perceived as being larger than the corresponding sizes of polystyrene throughout. There was no significant



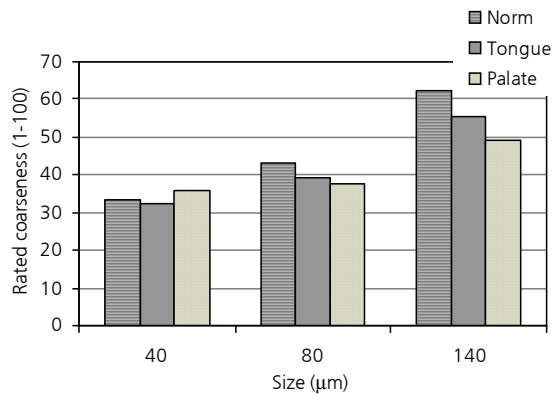
main effect of the viscosity of the medium, hence the subjects did not rate the size of particles differently, irrespective of the viscosity of the medium in which the particles were dispersed. In addition, there were no significant interactions.

Fig 2. Rated coarseness (1-100) for SiO₂ and polystyrene in various sizes with fitted second degree polynomials.

Table 2. Mean \pm SD coarseness ratings (1-100) as measured by direct scaling for all sizes, two materials (SiO₂ and polystyrene) and two viscosities (3 and 6 Pas).

Size (μm)	3 pas		6 Pas	
	SiO ₂	Polystyrene	SiO ₂	Polystyrene
Control	6.3 (\pm 6.2)	6.3 (\pm 6.2)	12.9 (\pm 6.8)	12.9 (\pm 6.8)
2	11.0 (\pm 13.8)		4.5 (\pm 4.9)	
40	31.0 (\pm 16.9)	28.3 (\pm 18.9)	32.5 (\pm 17.1)	23.0 (\pm 16.6)
80	43.0 (\pm 21.0)	44.6 (\pm 18.0)	33.7 (\pm 8.3)	38.7 (\pm 25.8)
140	61.6 (\pm 23.5)	50.4 (\pm 16.0)	49.9 (\pm 26.5)	12.9 (\pm 6.8)
180	66.5 (\pm 31.0)		60.6 (\pm 26.5)	
230		31.2 (\pm 26.0)		44.0 (\pm 23.4)

Influence of different oral parts in size perception



The size of particles significantly influenced the size perception ($p = 0.001$), where the subjects reported the sizes to be larger with increasing particle size. However, none of the two anaesthetic-regimes produced results significantly different to the control situation (Fig 3). There were no significant interactions between the treatments and particle size.



Fig 3. Rated size (1-100) of SiO₂ at three different sizes for the three treatments: no anaesthesia and topical anaesthesia applied to tongue or palate.

Forced-choice rankings

The sizes of the particles in the samples were ranked from fine to coarse. The results of the mean rankings are shown in Fig 4. Again, subjects were able to discriminate between the particle sizes in the samples, *i.e.* there was a significant main effect of size in the ANOVA ($p < 0.001$). Similar results were observed for both types of particles.

The means of the calculated scores were 11.6 ± 2.0 (range: 9-15) for SiO₂, and 7.8 ± 1.8 (range: 4 -10) for polystyrene. Z-tests indicate that the subjects were significantly better than chance in ranking the sizes of the particles, $p < 0.001$ for both SiO₂ and polystyrene.

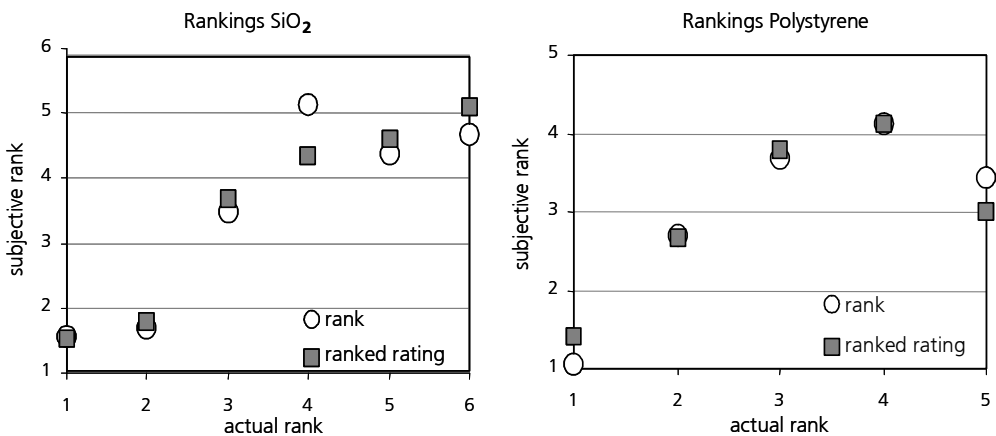


Fig 4. Subjective rankings for a. SiO₂ and b. polystyrene. Both the rankings from the forced ranking study and the ratings from the direct-scaling converted into rankings are included.

Relation between the two methods of size perception

Following the results of the paired t-test, there was no significant difference between the two methods ($p = 0.11$). Hence, the two methods (direct scaling and forced rankings) produce similar results.

DISCUSSION

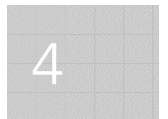
Three studies have been performed to investigate how different parameters affect size perception of particles: 1. The importance of type and size of particles and viscosity of the dispersion medium on size perception of particles were studied by rating the samples in comparison to anchor stimuli. 2. The relative importance of the different oral parts was studied by applying topical anaesthesia to the tongue or palate. 3. The effect of particle size and type on size perception was also studied by forced-choice ranking, where after the results of the two methods – direct scaling and rankings – were compared.

Direct-scaling

In the first study, the influence of particle size and type, in addition to the viscosity of the dispersion medium on size perception was investigated. The subjects' rating of particle size increased with increasing actual particle size, which is not surprising, but gives a good indication that subjects are able not only to perceive particles of the micron scale, but also to separate and classify the different sizes. The same effect was seen in the ranking study.

The type of particle also affected the size perception significantly. Hence, the two materials were not perceived in the same way, although the sizes were matched. The SiO₂ particles were reported to be larger than the polystyrene particles. A possible explanation for these differences could be attributed to the physical characteristics of the two materials. The SiO₂ particles are hard and irregular, while the polystyrene particles are round and soft. It also seems plausible that the larger polystyrene particles are indentable and “shapable” This suggests that the hard and irregular particles are perceived as being more present, whereas the soft and smooth particles are better “disguised” in the medium. One could hypothesize that this effect might be even stronger as the medium in which the particles are dispersed gets more viscous and particles need to be bigger, harder and more irregular to be sensed. A parallel can be drawn to particles of a different magnitude: A capsule of medicine can be very difficult to swallow with water only, but if placed in for e.g. mashed potatoes, it can be swallowed, hardly being noticed. It is however not likely that one can sense particles of the micron size, such as used in the present study, as single particles and hence these will probably not be sensed by pressure receptors with a certain receptive field. It seems more plausible that the particles are sensed in another way, perhaps as a friction or vibration (19;20). Assuming this is the case, this produces another explanation for the fact that the hard and irregular particles were perceived as being

larger, as they produce more friction *in vitro* (21). For micro-particles the sensation of size seems to be influenced by the degree of vibration, with a stronger sensation leading to a perception of a coarser particle size. This could again explain why the large (230 μm), indentable polystyrene particles were perceived as being smaller than even the particles of 80 μm . Previous studies have reported that hard and irregular particles are perceived as being rougher than particles of softer and smoother materials (11;14;22). The finding of the present study failed to prove the theory that a higher viscosity would mask the particles, since the viscosity did not show a significant influence on the perceived particle size. This is surprising since previous research has shown that the medium does have an effect on roughness perception (13). However, in this study we used only two viscosities, and even though the thick medium had a twice as a high a viscosity as the thin medium, this difference might not have been large enough to prove the point. Alternatively, both might have been thick enough to disguise some of the particles.



Influence of different oral parts

Topical anaesthesia applied to the tongue or the palate, did not have any influence on size perception of particles. This result was a bit surprising, since the superficial receptors were, though perhaps not fully, at least partly anaesthetized and we had expected this to affect the sensation of the particles. Especially since the samples were merely rubbed between the tongue and palate. Even so, the receptors signalling on size, whether this is done through measurements of vibrations or otherwise, seem not to have been affected by the anaesthesia, perhaps due to being situated in deeper layers of the tissue. It would be interesting to investigate oral size perception by excluding the tongue and palate, either by mechanical exclusion, or by a more potent anaesthesia. In this study, though, topical anaesthesia was chosen for the benefit of the subjects' comfort.

Relation between the two methods of size perception

Two methods of size perception; direct scaling and rankings were performed and the results were compared. The paired t-test reveals that there was no difference in scores between the two methods. In addition, there were very good correlations between the two methods. These results imply that the two methods, even though quite different, generate very similar results on the perception of size for both types of particle. The direct-scaling method, where the subjects received anchor stimuli which they had to remember for a relatively long time, depends on a well functioning memory of the subjects. On the contrary, during the size assessment of the ranking method, the subjects could compare the samples as many times as they wished and with all the other samples. The implication of these results is that both these methods can be used successfully for the assessment of oral sensitivity.

In conclusion, we have observed that the size and characteristics of the particle were of importance for perception of particle size, where large, hard and irregular particles were perceived as larger than soft and round particles were. The coarseness of such small particles is

suggested to be perceived through their type of mechanical stimulation, perhaps vibration. The viscosity of the dispersion medium failed to affect size perception in the present study.

Topical anaesthesia did not affect size perception, presumably because the particles were perceived by more deeply-set receptors.

The two methods of assessing size – direct scaling and forced rankings- produced very similar results in subjects with a well functioning memory.

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CHAPTER 5
RELATING PARTICLES AND TEXTURE
PERCEPTION

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ABSTRACT

Practically all foods contain particles. It has been suggested that the presence of particles in food may affect the perception of sensory attributes. In the present study we investigated the effect of size and type (hardness and shape) of particles added to a CMC based vanilla custard dessert. The two types of particles included in the study were silica dioxide and polystyrene spheres, varying in size from 2 - 230 μm . Eighteen trained adults participated in the study. They rated the sensation of 18 sensory flavor and texture attributes on a 100 point VAS-scale. The results indicate that the addition of particles increased the sensation of roughness attributes and decreased the ratings of a number of presumably favorable texture attributes (smoothness, creamy, fatty and slippery) significantly. These effects increased with increasing particle size up to particles around 80 μm . Surprisingly, even particles of 2 μm had significant effects: they increased perceived rough lip-tooth feel, and decreased slippery lip-tooth feel and smoothness of the product.

In a separate study on size perception the same stimuli were used. By sampling the stimuli between the tongue and palate, subjects rated the size of the particles on a 100 point scale in comparison to anchor stimuli containing no particles and particles of 250 μm . These results were correlated with the sensory results. Significant positive correlations were observed among size perception and smoothness and fattiness. Rough sensation was negatively correlated with size perception, indicating that beyond a certain particle size, even if the particles are strongly sensed and present, subjects no longer include the sensation of the particles in their assessment of texture perception. This suggests that in order for particles to have an effect on texture perception, it is important that they are sufficiently small.

In conclusion, particles added to a product induce large effects on texture sensations and texture sensation is related to size perception.

INTRODUCTION

Practically all food contains particles. While some are obviously present such as pits in berries, others are small, or soft and hardly noticeable, such as the quite large, but soft starch granules in a pudding, or oil droplets in mayonnaise. Hence, food particle sizes vary from very large to sub-micron size. The concentration of particles varies from the single seed in a grape to large volumes affecting texture – here we will focus on particles in the food structure, ranging from 2-230 μm . The minimum particle size that can be detected by the palate is 25 μm , as viewed by the confectionery literature (1). If particles in chocolate are below this size, the optimum smoothness is achieved (2). Particles in chocolate are however not very hard and though irregular in shape, they do not have sharp edges. Hard and irregular particles, e.g. alumina produce a gritty effect even at particle sizes around 10 μm (3). Larger particles will produce a very gritty sensation and are sufficiently hard to scratch the enamel surface (4). Imai et al. (5;6) reported that concentration, dispersion medium and particle size were all important factors contributing to perceived grittiness. The proportion of people who perceived grittiness grew with increasing particle size and increasing particle concentration. They also observed that perceived grittiness decreased as the viscosity of the dispersion medium increased, and as the oil droplet size in oil-in-water emulsions decreased. Kilcast and Clegg (7) investigated the effect of particle size and concentration on perceived creaminess of soft model systems containing solid particles. They found reduced creaminess with larger particles size and higher concentration.

Tyle (8) investigated the effect of shape, size and surface properties of particles on the rough sensation, sensitivity, and acceptability of a product. He found that hard particles with sharp edges produce gritty sensations at smaller sizes than soft and round particles do.

The conclusion of the above studies is that large, hard, sharp particles in a low viscosity medium seem to produce a more rough, gritty and unpleasant sensation than small, soft and smooth particles in a higher viscosity medium.

Previously, it was demonstrated that two sensory dimensions, one running from perceived thickness to perceived melting, and one from rough related attributes to perceived creamy/fatty, could summarize the sensory space for vanilla custard dessert (de Wijk et al, 2003). As creaminess is a rather important attribute in the appreciation of soft products, previous studies have attempted to unravel creaminess (9). These studies have demonstrated that creaminess is a complex attribute strongly related to thickness (10) and smoothness (11), as well as to a flavour or taste attribute (7;12). This was also observed in modeling analyses, where creaminess of vanilla custard dessert was predicted from a combination of flavors (creamy- and fatty flavor and absence of bitter/chemical and sickly flavors), thickness, fattiness, and (absence of) roughness ratings (13). As two of the sub-attributes are smoothness and lack of roughness, an addition of particles to the product could be a relevant way to artificially induce the sensation of roughness and hence, reduce creaminess. It is suggested that very small particles (in the range of 0.1-3 μm (14), below 4-7 μm (7)) even might increase smoothness and creaminess. It can be questioned whether this is true for all types of particles. As Tyle and



others have observed and suggested, the type, i.e. shape and hardness, of the particle is of importance. To test the effect of shape and hardness on texture perception, we used silica dioxide, which is hard and has sharp edges and spheres of polystyrene (Dynoseeds®) in various matching sizes.

In order to be able to manipulate the viscosity of the stimuli, we chose to manufacture the custard stimuli in the lab instead of using commercial products. Carboxy methyl cellulose (CMC) was chosen as the thickener for a number of reasons even though it is not a commonplace thickener in commercial custards. CMC thickens during cold mixing. This is favorable to hot mixing as the latter is difficult to standardize in a simple laboratory. Another beneficial characteristic is that CMC based stimuli are not broken down by the salivary enzyme α -amylase, assuring that the stimuli retain their in-mouth viscosity longer during the assessment.

Subjects are highly diverse in their ability to assess the size of an object in the mouth (15) (Engelen et al, submitted). While some perceive the size correctly, others over- or underestimate the size of the object when matching the size in the mouth with a visual reference set. In addition, subjects are also diverse in their sensory ratings. In spite of assessing the same stimulus, subjects report the stimulus to be sensorially different, texture-wise. Taking this diversity in texture perception and oral sensitivity to size into consideration, we hypothesized that the difference in sensory ratings could be related to the ability to rate the size of particles.

The purpose of this study was two-fold: Firstly, we studied the general effect of added particles on texture perception, and the effect of particle size and type in specific. Secondly, we were interested in the relation between subjects' particle size perception, and their perception of texture in custard dessert.

METHODS AND MATERIAL

Subjects

Eighteen (13 female and 5 male) trained adult panelists participated in the first study. Their age ranged between 20 and 36, average age was 23 years. The subjects were selected on the basis of a well functioning smell and taste perception. The subjects gave informed consent and were compensated for their participation. Each subject was always tested at the same time of the day. Eleven subjects were measured for both studies 1 and 2.

Stimuli

Dispersion medium

Custard dessert was prepared in the laboratory by blending 8.5 g Carboxy Methyl Cellulose (Akucell AF3295 Akzo Nobel, Amersfoort, the Netherlands), 62.5 g sugar and 1.5 g vanilla flavour (3912 Danisco) and thereafter add the dry blend to 1 liter of commercial full-fat (3 %

milk (AH, www.ah.nl) during mixing. The custard was mixed in a professional mixer for 25 minutes. 5 minutes before the end, 1 ml of yellow food colorant (Egg yellow, Supercook, Leeds, UK) was added per liter of custard to enable the color to mix in thoroughly. The custard desserts were prepared on the day of evaluation and stored and administered at 10°C, which is the normal serving temperature in the Netherlands.

Particles

Silica dioxide (2.5 g/cm³; U.S. Silica company, Ottawa, IL) (Fig 1a) in different size grades was ordered from the manufacturer. These grades were sieved into discrete classes; 20-50 μm, 50-100 μm, and 100-150 μm (Interlab B.V., Etten-Leur, Holland). The median particle sizes of these classes were determined by Coulter laser diffraction to be 40, 80 and 135 μm, respectively. In addition, the grade Min-u-sil 5, had a median particle size of 2 μm, as specified by the manufacturer. Spherical polystyrene particles (1.10g/cm³; Dynoseeds®, Polymer-systems.com) (Fig 1b) were ordered from the manufacturer in the discrete sizes 40, 80, 140, and 230 μm. In table 1, the sizes and types of particles included in the study are given. Imai et al (5) have shown that the concentration of particles is of importance for the perception of grittiness and probably also of other texture attributes. It can be hypothesized that the number of particles is crucial, as opposed to the weight of the particles. Therefore we chose to add approximately the same

number of particles of both types of particles to the dispersion medium. Since the density of silica is more than twice as high as of the polystyrene particles, we added 5% weight of silica and 2.3 % weight of polystyrene particles to the custard dessert. The particles were mixed well with the custard and placed in 25 ml containers prior to sensory evaluation.

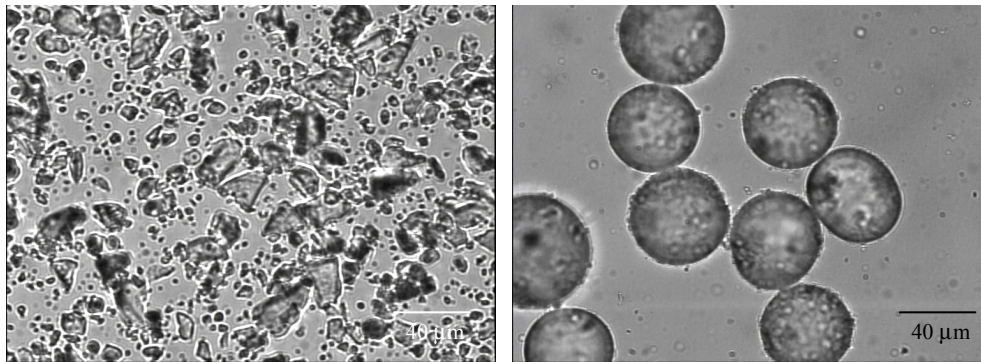


Fig 1. Micrographs of a. silica dioxide and b. polystyrene samples with a mean diameter of 40 μm.



Table 1. Sizes of the two types of particles (silica dioxide and polystyrene) included in the study.

Mean Size (μm)	0	2	40	80	140	230
SiO ₂	Control	X	X	X	X	
Polystyrene			X	X	X	X

Study 1: Sensory testing

Attributes

Eighteen sensory attributes, including odours (almond and synthetic/sickly), flavours (vanilla and bitter/chemical), tooth-lip feel (astringent and smooth), mouth-feel (temperature, thickness, airy, melting, prickling, smooth, heterogeneous and creaminess), and after-feel (coating, sticky, fat and astringent), were rated for the custard. Tooth-lip feel is the sensation that arises when rubbing the tongue against the upper lip and upper teeth, and after-feel is the sensation remaining after swallowing. The definitions of the rated attributes are given in Table 2. These attributes were selected as a representative sub-set from a set of 35 attributes developed previously for vanilla custard desserts by a Quantitative Descriptive Analysis (QDA) panel (9;16).

Procedure

The subjects were seated in sensory booths with appropriate ventilation and lighting. During 2-hour sessions on two separate days, subjects were presented with triplicates of all the stimuli. The custard was first sniffed, after which the odour attributes were rated. Next, stimuli were ingested with a spoon and ingested custard was rated on flavour and mouth-feel attributes. Subjects had been instructed to process the stimuli in the mouth in their normal way, however they were asked to refrain from chewing the stimuli, or in any way put it between the teeth. Subjects kept the stimuli in the mouth during 4-5 seconds, which previously had been observed to be the time they normally kept the stimuli in the mouth while assessing the same group of attributes (unpublished data). Finally, the custard was swallowed and four after-feel attributes were rated. Acquisition of the subjects' responses was done by computer on a 100-point VAS response scale. Panel testing took place at the sensory facilities of TNO-Nutrition and Food Research in Zeist, the Netherlands.

Table 2. A list of the attributes included in this study and their definitions sorted by the functional types: flavour (fl), lip-tooth feel (lt), mouth feel (mo), and after feel (af).

Attribute	Definition
Flavour/taste (fl)	
Bitter/chemical	Degree to which the taste of a product is bitter.
Vanilla	Intensity of vanilla flavour.
Lip-tooth-feel (lt)	
Rough	The rough sensation elicited when rubbing the tongue against the front teeth and inside of the lip after the first contact with the product
Slippery	The slippery sensation elicited when rubbing the tongue against the front teeth and inside of the lip after the first contact with the product.
Mouth-feel (mo)	
Temperature (cold - warm)	Foods may elicit different temperature sensations while presented at the same physical temperature. Sensation is sensed during first contact between food and tongue.
Thickness	Represents the thickness of the food in the mouth after the food is compressed via up- and down motions of tongue against palate.
Airy	Food is perceived by tongue as airy/foamy and disintegrates easily after the food is compressed against the palate.
Melting (slow - quick)	A food becomes thin in the mouth and spreads throughout the mouth at different rates
Prickling	A tingling feeling sensed by the tongue typically associated with slightly carbonated soft drinks.
Smooth	Degree in which the food contains granules detected by moving the tongue parallel to palate.
Heterogeneity	Food is sensed simultaneously as thick and thin (or "cloudy" or "flocky") in the mouth while food is mixed with saliva. Various parts of the food seem to melt at different rates.
Creamy	Range of sensation typically associated with fat content such as full and sweet taste, compact, smooth, not rough, not dry, with a velvety (not oily) coating. Food disintegrates at moderate rate.
After-feel (af)	
Creamy	A velvety (not oily) coating remaining after swallowing.
Sticky	The residual custard leaves a sticky feeling in the whole mouth which is difficult to remove.
Fatty	Food leaves a fatty/oily feeling in mouth after swallowing.
Rough	Food leaves a rough taste and feeling in the mouth.



Study 2: Size perception

Subjects were instructed to place the stimuli on the tongue and rub the tongue against the palate in order to identify particles. The subjects received two anchor stimuli: one containing no particles, the subjects were informed that these were the smallest particles they would receive and these were to be rated at the beginning of the line scale; the other anchor stimulus containing silica particles of 250 μm , the instruction was that these were the largest particles included in the study and were to be rated at the far end of the scale. The anchor stimuli were redistributed twice times during the experiment in order to recalibrate and refresh the subjects' memory. Five stimuli with added SiO_2 (2, 40, 80, 140, 230 μm) in addition to a control were administered to the subjects in duplicate during a two-hour session separate in time and location from the sensory testing in order to ensure that the two parts of the study were not interfering. The assessment took place on a one to one basis, with the sole objective of rating the size of the particles. Subjects' responses were scored on a 100-point VAS response scale.

Data processing and analysis

Sensory data were collected and analyzed by FIZZ software (1998, Biosystèmes, Couternon, France). Repeated-measures ANOVAs were performed with Greenhouse-Geisser as correction factor on data averaged across triplicates (SPSS 9.0 SP 4M, SPSS inc., Chicago, IL). Size and type of particle were included as within-subject factors. The same software was used to perform Spearman's correlations on perceived size and perceived texture. $p < 0.05$ was considered significant.

RESULTS

Sensory testing

Table 3 depicts the mean sensory ratings for the two types and five sizes of particles. The significant effects of type and size of the particles on sensory ratings are indicated for the various attributes.

Fig 2 illustrates the effect of added particles of different sizes in comparison to the control (particle free custard) for the significantly affected texture attributes. 0% indicates that there was no difference in comparison to the control custard, i.e. the addition of particles had no effect. Positive values represent the percentage increase in sensation compared to the control custard and consequently, negative values represent percentage decrease in comparison to the control.

Table 3. Means and (SD) of the sensory ratings for each attribute for the control stimulus and the stimuli with added particles of varying size and type.

Size	Type	bitter/chem-fl †	vanilla-fl †	rough-it *	slippery-it *	cold-mo	thickness-mo	airy-mo	melting-mo	fatty-mo *	prickling-mo	smooth-mo *	heterogeneity-mo *	creamy-mo *	creamy-af	sticky-af †	fatty-af *	rough-af †*
0	Control	43.8 (25.6)	34.0 (16.2)	30.3 (19.8)	48.1 (18.8)	36.8 (15.2)	49.3 (19.3)	49.3 (20.2)	40.2 (22.3)	46.9 (21.3)	18.1 (12.2)	50.9 (25.1)	35.8 (23.0)	42.5 (20.1)	37.1 (20.5)	46.3 (20.3)	42.7 (23.6)	25.1 (17.8)
2	SiO2	47.8 (25.6)	29.1 (15.6)	37.6 (22.0)	40.5 (19.8)	41.0 (16.9)	50.3 (18.1)	45.2 (20.5)	38.0 (21.5)	43.6 (21.5)	17.0 (10.3)	41.3 (24.7)	46.7 (27.2)	42.4 (17.5)	37.5 (21.4)	44.2 (19.6)	41.3 (20.6)	25.5 (17.3)
40	SiO2	47.0 (24.4)	31.5 (17.8)	41.9 (23.5)	39.0 (18.8)	37.9 (16.0)	47.7 (20.1)	46.3 (19.6)	40.0 (21.1)	40.5 (20.8)	20.1 (15.3)	37.0 (25.6)	40.6 (22.4)	39.2 (17.7)	38.0 (21.4)	38.1 (18.0)	34.5 (19.6)	35.9 (18.0)
80	PolyS	48.0 (24.5)	28.4 (14.1)	39.2 (23.5)	39.6 (19.6)	37.7 (15.3)	47.2 (19.2)	45.0 (19.6)	41.0 (22.9)	37.9 (19.3)	21.4 (13.7)	40.8 (25.2)	36.3 (22.5)	38.3 (20.7)	36.6 (19.9)	41.0 (17.8)	36.0 (20.5)	32.1 (19.5)
140	SiO2	44.0 (24.5)	32.1 (18.0)	46.1 (26.0)	36.9 (20.2)	37.3 (16.1)	47.9 (17.7)	44.1 (21.1)	41.7 (22.8)	37.7 (19.8)	21.1 (16.0)	35.3 (26.2)	43.1 (23.7)	39.3 (19.9)	33.3 (19.6)	39.6 (17.0)	32.7 (18.8)	40.1 (22.7)
230	PolyS	47.0 (26.2)	28.5 (16.2)	41.0 (25.0)	36.5 (19.5)	40.9 (16.5)	43.6 (19.0)	43.7 (19.4)	41.2 (23.0)	42.1 (20.4)	23.0 (17.7)	40.2 (25.5)	41.8 (22.2)	40.0 (20.5)	36.8 (19.4)	42.1 (19.1)	37.0 (21.3)	31.2 (18.4)
0	SiO2	47.0 (23.7)	29.4 (15.2)	42.1 (23.0)	36.7 (19.6)	35.3 (14.3)	47.2 (21.2)	47.5 (19.7)	37.8 (22.5)	36.8 (19.8)	21.1 (15.5)	36.4 (24.7)	40.9 (22.4)	40.4 (21.4)	36.0 (19.2)	38.2 (18.5)	34.9 (21.5)	39.2 (21.9)
40	PolyS	47.4 (27.0)	29.1 (15.3)	37.8 (23.9)	42.5 (19.1)	38.0 (15.5)	46.1 (19.1)	47.4 (17.8)	38.5 (22.4)	39.6 (19.9)	21.5 (16.1)	39.2 (24.9)	42.5 (23.8)	39.6 (18.4)	37.2 (18.6)	44.7 (19.9)	36.8 (21.3)	31.7 (21.1)
80	PolyS	48.1 (26.3)	28.8 (15.3)	35.1 (23.7)	37.7 (22.0)	35.8 (14.6)	48.9 (21.1)	44.9 (20.1)	38.6 (23.2)	42.4 (20.5)	19.8 (15.2)	36.8 (23.5)	41.8 (25.8)	36.6 (19.3)	37.6 (21.2)	41.4 (21.3)	37.5 (22.5)	26.4 (17.2)

† significant effect of type of particle, p<0.05
 * significant effect of particle size, p<0.05



We observed a large effect of added particles on a considerable number of texture attributes (Fig 2). Especially the size of particles had a substantial effect. The sensation of rough lip-tooth feel ($F(1.2, 20) = 6.8, p = 0.013$) and rough after feel ($F(2.2, 36) = 8.3, p = 0.001$) increased with the addition of particles with a maximum effect obtained for particles around 80µm, where after the sensation decreased with larger particles. Presumably favourable attributes, including slippery lip-tooth feel ($F(1.8, 28) = 8.0, p = 0.002$), smooth ($F(1.3, 20.7) = 7.5, p = 0.008$), creamy ($F(2.2, 35) = 10.9, p < 0.001$), and fatty mouth feel ($F(1.9, 30) = 7.8, p = 0.002$), and fatty after feel ($F(2.2, 35) = 7.4, p = 0.002$) decreased with the addition of particles. Again, the effect increased with increasing particle size up to particles of around 80 µm, however, the sensation leveled off with larger particles. It is interesting tonote that particles of 2µm affected the attributes smooth mouth feel ($F(1, 16) = 7.0, p = 0.017$), rough lip-tooth feel ($F(1, 16) = 9.0, p = 0.017$) and slippery lip-tooth feel ($F(1, 16) = 8.3, p = 0.011$) to a large extent.

The type of particle had limited effect on the perceived texture sensations. Silica dioxide produced a stronger sensation of rough after feel at sizes 80 and 140 um than did the polystyrene particles ($F(1, 16) = 8.4, p = 0.011$)

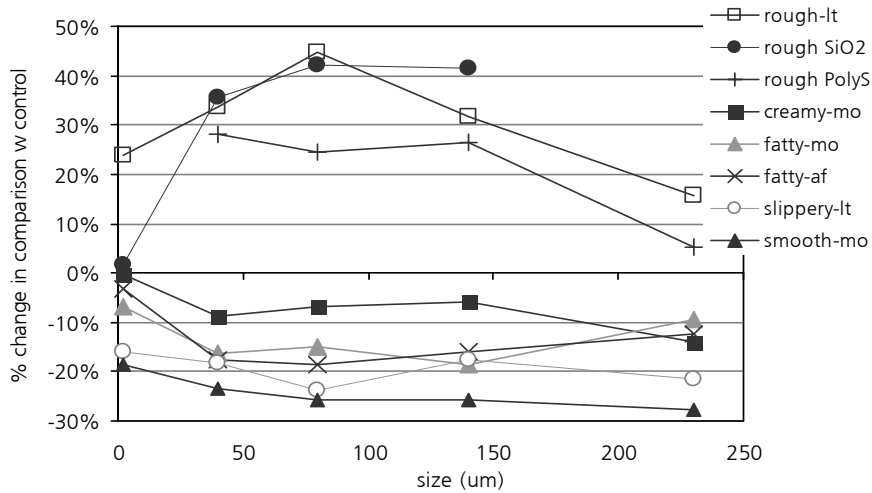
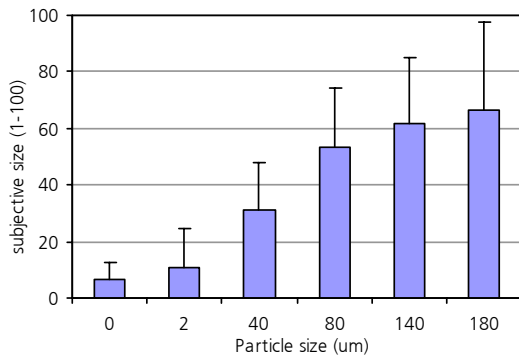


Fig 2. The change in ratings (%) in comparison with the control. The attributes shown in the graph were the texture attributes for which an addition of particles had a significant effect, $p < 0.05$.

Correlation between size, and texture perception



The results show that subjects were good at perceiving the size of particles, hence the perceived particle size increased with increasing particle size (Fig 3).

Fig 3. Means of the size perception of the control stimulus and the stimuli with added SiO₂ particles of varying size. Error bars indicate \pm SD

There was a significant negative correlation between the perceived particle size and the sensation of roughness ($R=-0.72$, $p=0.012$). (In Fig 4a an example is depicted for particles of 40 μm). This suggests that subjects, who were sensitive and perceived the particles as being large, reported the same stimuli to have less rough after feel. Smooth mouth feel ($R=0.71$, $p=0.014$) (Fig 4b depicts an example for particles of 80 μm) and fatty after feel ($R=0.72$, $p=0.013$) were positively correlated with the perceived particle size. Hence subjects, who perceived the particles to be large, also reported strong sensations of smoothness and fattiness.

Fatty after feel was also significantly and positively correlated with size perception and the graph was similar to the one for smoothness. This implies that subjects who were sensitive and perceived the particles as being large, reported the same stimuli to have less rough after feel and being more smooth and fatty.

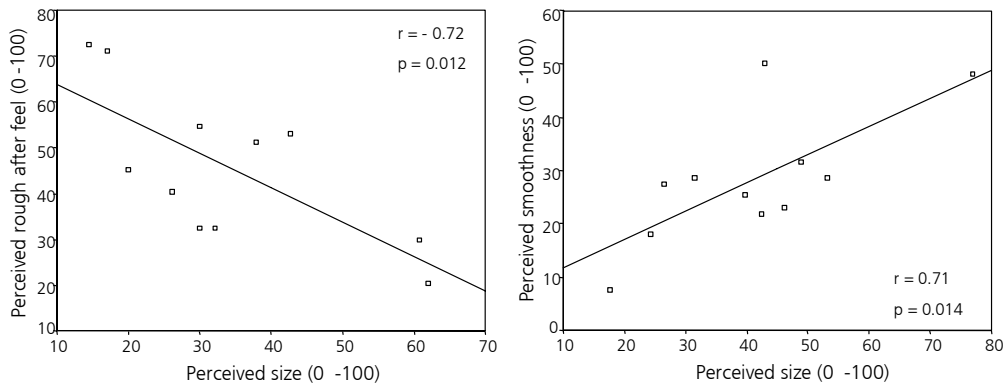


Fig. 4. Correlations between size perception and texture perception for the attributes rough after feel (4a) and smoothness (4b).

DISCUSSION

In this study the effects of particle size and type on sensory perception were investigated. In addition, the relation between perceived particle size and sensory perception was studied.

In a pilot study the effect of particles on viscosity was investigated. As no significant differences in viscosity could be observed between the control and any of the stimuli with added particles, the conclusion was that any possible effect on the viscosity of particles at the concentrations we used could be neglected. This is of importance, since Imai et al., (7) have shown that the viscosity of the medium into which particles were mixed could affect the perception of textural characteristics, e.g. grittiness.

The type of particle was shown to have only limited effect on texture perceptions. Rough after feel was stronger for stimuli with added silica than with added polystyrene at medium and larger sizes. This effect may be explained by the shape of the particles. During oral manipulation of the stimuli, the particles were dispersed in the medium and this medium might have masked the sharp edges of the silica particles. During deglutition, most of the medium is swallowed, leaving a small amount of particles behind in the oral cavity. Since there then was no medium to soften and disguise the particles, the sharp edges of silica particles “scratched” the oral mucosa and hence were noticed more strongly. In addition to being round, the



polystyrene particles also get more flexible and indentable at larger diameters, resulting in a less hard and rough sensation. Possibly the particles will deform if they are equally soft or softer than mucosa. Vice versa, if the particle is harder than the mucosa, then mucosa will deform and mechanoreceptors will be triggered. Imai et al. (17) reported that graininess is enhanced for particles with a “solid structure”, hence particles which are not easily deformed, have low water absorption rate and low water solubility.

It would be interesting to investigate this matter further.

In the present study even particles of 2 μm in diameter had an effect on texture sensations: rough lip tooth feel increased with 20 % in comparison to the control situation, and slippery lip tooth feel and smooth mouth feel decreased with the same magnitude. This suggests that the receptors in the oral mucosa are able to sense particles that small. Even though roughness and smoothness were strongly affected, these small particles failed to affect the perception of creaminess. This was quite a surprising observation, since the few reports in the literature on particles of that size, suggest that small particles can enhance creaminess (7;14). Creaminess is a combination of a lack of roughness and presence of smoothness, in addition to viscosity, fatty and flavour components. For that reason one would expect creaminess to be affected when roughness and smoothness are. As viscosity and flavour were kept close to constant, this reveals that there might yet be another aspect to creaminess still to be discovered.

The attributes affected by the addition of particles, are thought to be driven by their surface-related properties. The sensation of roughness is suggested to be independent of the thickness of the stimulus layer, and could in theory be sensed just by covering the mucosa with a very thin layer of stimulus. The same would be true for smooth and fatty and to certain extent creaminess. Extensive studies in this laboratory aiming at relating a wide range of rheological measurements with sensory data (Janssen et al., in prep.) supports this idea. The results have shown that the attributes melting, roughness, fatty-mouth feel and fatty-after feel are relatively poorly predicted by rheological measurements. Hence these attributes seem to reflect primarily surface-related properties as opposed to bulk properties of e.g. the attribute thickness. Creaminess however has previously been observed to be a complex attribute (7;11;13) and correlates to some rheological measurements as well. If the sensation arising from the particles, leading to an increased roughness and decreased smoothness etc. is surface-related, the mechanism is probably related to friction. Evidence supporting this idea has been collected in our laboratory (18). *In vitro* friction measurements on custard with saliva added to it, in order to mimic the *in vivo* situation, have been correlated with oral perception of the same stimuli. A positive correlation was observed, indicating that as friction increases, so does rated oral roughness. Furthermore, increased fat content resulted in lower friction, lower sensations of roughness, and higher sensations of creaminess, suggesting that lubrication is one of the predominant mechanisms by which fat reduces sensations of roughness.

What type of mechanoreceptor that would pick up this type of modality (friction) is not quite clear. One could hypothesize that friction would be a type of vibration. Hollins *et al.* reported that for fine surfaces (below 100 μm) assessed by the finger, vibration is the main cue to texture

(19). They confirmed this finding by demonstrating that a vibrating surface induced a less smooth perception than did a stationary, as sensed by the finger (20). However, it is believed that the oral mucosa lacks Pacinian corpuscles, responding to vibration (21). Johansson *et al.* (22) showed that oral mucosa is innervated mainly by slowly adapting units. In accordance with this, the information on roughness perception is suggested to be conveyed by the slowly adapting (SAI) system (23) (24;25). The latter results are based on stimuli of around 1 mm assessed digitally, so whether they also hold for particles with diameters ten to hundred times smaller sensed in the mouth, is not clear. Conversely, Trulsson and Essick (26) reported that two thirds of the superficial tactile units of the tongue were rapidly adapting and only one third slowly adapting. The response properties of these were found to be similar to RA I, SA I and SA II tactile afferents of glabrous skin in human hand. These superficial units, which have very small receptive fields and low thresholds, responded vigorously when the tongue was moved into physical contact with other intra-oral structures. The RA I receptors are sensitive to vibrations up to 50 Hz and could hence probably sense mechanical roughness (Trulsson, personal communication). These studies demonstrate that various oral structures have different innervations.

An interesting question to address is where in the mouth the sensations, leading to a perception of roughness are sensed. During a study in this laboratory, in which the influence of the palate in texture perception was investigated, custom made plastic palates were made for each of the subjects. The same stimuli were assessed during the normal situation and while the plastic palate was in place. The results show significant attenuation of sticky and rough after feel, when excluding the sensation from the palate. This implies that perception of roughness arises from a combination of input from the palate and tongue.

One could expect that subjects who are relatively sensitive to particle size, i.e. they sense very small particles and perhaps even overestimate particle sizes, would also display high sensitivity to roughness. Therefore, the observed correlation between particle size perception and texture perception, where subjects who overestimated particle size rated the stimuli to be smoother and less rough may be counterintuitive. One would expect those subjects to experience a stronger sensation of roughness than subjects who perceived the particles as being smaller. However, if a subject perceives particles in a stimulus as separate particles as opposed to part of the bulk, he/she might not consider the particles to increase roughness. Accordingly, subjects who perceived the same size of particle to be small, reported the stimulus to be rough. A plausible explanation can be envisioned in the following example: An almond in the porridge is obviously not a constituent of the porridge itself, but added to it, and can easily be singled out and does accordingly not affect the texture sensation of the porridge. If the almond was ground into smaller parts, there would be a break point where the almond particles would be considered part of the porridge. This cut-off point in size is not discrete and is probably different for different individual and dependent on the medium.

The opposite correlation was seen for fatty and smooth, and the same explanation could be applied for these two attributes. As long as the particle size is larger than the individual break



point, the particles seem to have a negligible effect on perceived texture, if the concentration of particles is kept limited.

In conclusion addition of particles to custard dessert had affected the perceived texture strongly. While type of particle played a minor role in this study, the size of the particles was of significance. The mouth was observed to be highly sensitive even to very small particles and silica particles of 2 μm had a strong effect on smooth and rough sensations. There were correlations between size sensitivity and texture perception, where sensitive subjects were more able to exclude the presence of particles from their perception of texture. This study invites to further research into the continuous relation between roughness and creaminess.

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CHAPTER 6

THE ROLE OF INTRA-ORAL MANIPULATION
IN THE PERCEPTION OF SENSORY ATTRIBUTES

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ABSTRACT

To gain insight into the effect of oral processes on perception, we defined a set of five specific oral manipulations and investigated their effects on the perception of low and high fat versions of two semi-solid foodstuffs, vanilla custard desserts and mayonnaises. Behavior modifications ranged from simply placing the stimulus on the tip of the tongue to vigorously moving it around in the mouth. Sensory ratings for mouth-feel and flavor attributes were made 5 s after placing the stimulus in the mouth, and after-feel attributes were rated immediately after swallowing. Most attributes showed a similar pattern, with lowest attribute ratings where the tongue's movement was restricted and gradually increasing ratings with increasing complexity of the tongue movements. An individual's normal oral processing behavior typically resulted in the most intense sensations of flavor and mouth-feel. Residence time for all mouth-feel attributes, except prickling, was determined by the time required for tongue movements. The exact tongue movements required for sensations appeared to be related to food groups and individual foods, rather than to specific mouth-feel attributes.

INTRODUCTION

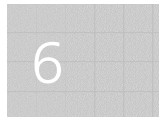
Once placed in the mouth, food is subjected to a complex series of manipulations by the tongue, teeth, lips and cheeks, during which it is converted into a form suitable for swallowing. Sensory assessments of food begin prior to ingestion when the food is seen, handled and smelt (1), and continues in the mouth to assess taste and texture during oral processing and swallowing. Some sensations persist after swallowing and are termed after-feel.

Sensations are gathered during each phase of oral food processing. Initial perceptions occurring at low shear rates include attributes related to touch (e.g., perceived homogeneity based on the presence, size, and shape of particles), and those requiring only small deformations (elasticity, stickiness to the palate, and viscous behavior) (2). Next, the food structure is broken down during the first chews (solid food) and the food mixed with saliva to form a coherent bolus (solid and semi-solid food) (3). During this phase, attributes related to the physical deformation and breakdown properties (e.g. hardness, softness, brittleness, plasticity, crispness, and sponginess) are detected (4;5). As more saliva is added to the bolus, attributes are perceived that relate to the physical structure (e.g. smoothness, lumpiness, and pastiness), consistency (e.g. creaminess and wateriness), and adhesion to the palate (e.g. stickiness).

Hutchings and Lillford (6) modeled the breakdown over time of various foods during oral processing. Key elements of their model are the mechanical breakdown of food structure and the degree of lubrication effected by the saliva and by the moisture and fat in the food. Lubrication was also the proposed underlying mechanism for a sub-set of the mouth- and after-feel attributes generated by a quantitative descriptive (QDA) panel for vanilla custard desserts (7). The other sub-set consisted of the attributes melting and thickness, which are probably related to the viscosity of food and its reduction through chemical and mechanical breakdown.

Information on details of the oral process is a prerequisite if physico-chemical measurements of foods are to be made under realistic conditions. For example, apparent viscosity, the stimulus property responsible for thickness sensations, depends on the shear rate applied in the mouth since most foods are non-newtonian. These shear rates vary from 0.1 to 1000 s⁻¹ (8). Hence, in order to measure apparent viscosities instrumentally under realistic conditions, they must be measured at shear rates similar to those found in the mouth.

Details of oral processes have been gathered primarily for solid foods using a wide variety of techniques, ranging from observations of muscle activity (9), jaw movement, particle-size distribution (10), the mixing of two color chewing gums (11), bite mark analysis of expectorated wax-wafers (12), facial movements (13) and direct observation by video-fluorography (14).



Common findings are that the following steps are involved in oral processing of solid food: 1) food is placed onto the anterior 1/3rd of the tongue; 2) the tongue is elevated, compressing the food against the palate; 3) the tongue is depressed, transferring solid foods to the post-canine teeth; 4) comminution; 5) swallowing; and 6) clearance. For semi-solids, oral processes are less well characterized, possibly because those processes require primarily tongue movements, which have proven to be difficult to monitor instrumentally without restricting masticatory movements.

Data on oral processes have also been gathered using introspective techniques. Engelen and van Doorn (15) identified four basic feeding styles for two semi-solid foods, custard and mayonnaise. Subjects were asked to describe chronologically, in their own words or by means of diagrams, what they did after placing the food in the mouth. Subjects were categorized into four groups; simple (50%), taster (20%), manipulator (17%) and tonguer (13%). “Simple” subjects placed the food on the front of the tongue, raised its tip to the palate to form a seal with the sides of the tongue against the teeth, then retracted the tongue and swallowed the food. “Tasters” first moved the food backward in the simple manner described above, but additionally made a series of short sucking movements against the palate before swallowing. Sometimes, tasters described transporting the food via the cheeks to the back of the mouth. “Manipulators” described a wide variety of behaviors, sometimes chewing with the incisors and allowing the food to flow into the buccal sulcus and/or chewing between the molars. “Tonguers” made back and forth and sideways movements of the tongue against the palate.

Certain attributes, such as coldness and thickness, can be assessed soon after ingestion. Others, such as smoothness and prickling, require longer residence times (7). Factors that may determine the temporal order in which attributes are assessed include the number and complexity of the manipulations required to position the food relative to the receptors and the time needed for the sensation to reach full strength. In this study, a new approach to gathering data on the relation between oral movements and attributes was explored. Rather than investigating oral processes during normal oral processing via instrumental or introspective methods, oral movements were experimentally modified and their effects on flavor, mouth- and after-feel sensations evaluated. Behavior modifications were selected based on common findings from the studies described above.

METHODS

Subjects

Twenty-one subjects (aged between 21 and 34 yrs) participated in the study using vanilla custard desserts, and fifteen in the study using mayonnaises and dressings (aged between 22 and 31 yrs). Most of the subjects participated in both studies. All subjects had previously been screened for olfactory and taste disorders and had received extensive training in the description of sensory mouth- and after-feel attributes for semi-solid foods. The subjects were paid for their participation. Testing took place at the sensory facilities of TNO-Nutrition and Food Research in Zeist, The Netherlands

Stimuli

Commercially available full (3.0% fat) and low fat (<0.5%) vanilla custard desserts (AH vanille vla, Albert Hein Corp., Zaandam, The Netherlands and Euroshopper vla, Creamex Corp., Rijkevoort, The Netherlands), and full (72% fat) and low fat (36% fat) mayonnaises (Calvé full and half-full mayonnaise, Van der Bergh Corp., The Netherlands) were used in both studies.

Procedure

Panelists were seated in sensory booths with appropriate ventilation and lighting. Subjects were trained over four 2-hr sessions during which they were presented with samples of custard desserts and mayonnaises using a sub-set of sensory attributes previously generated by another panel (Table 1) (7). Panelists assessed sensory attributes in the chronological order in which they are perceived (as established by the previous panel). Subjects were then trained to perform the oral behaviors described in Table 2.

Testing took place over two 2-hour sessions for each product group. Samples were presented in random order with instructions as to which manipulation the subject was to perform. Three replicates were performed for each experimental condition. The attributes appeared by category on a monitor placed in front of each panelist, listing attributes on the left with a 100-point response scale, anchored at the extremes, on the right (FIZZ Biosystemes, 1998). Panelists used a mouse to indicate the perceived intensity of each attribute on this scale. Odor attributes were scored prior to placing the product in the mouth, mouth-feel attributes were assessed 5 seconds after taking the product into the mouth, and after-feel attributes were rated immediately after swallowing. The stimuli were presented at an average rate of one stimulus per 5 min. Each stimulus was taken into the mouth after which the four mouth-feel attributes were rated in the fixed order. Finally, the stimulus was swallowed and two after-feel attributes were rated. Acquisition of the subject's responses was done by computer using FIZZ software.

Statistical analysis

The effects of modified behaviors on sensory attributes were analyzed using a repeated measures ANOVA (SPSS, SPSS Inc) with the Huynh-Feldt value as epsilon, carried out on the sensory data averaged across replicates.

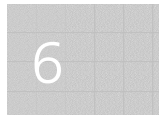


Table 1. List of descriptive terms related to odor, flavor, mouth-feel and after-feel for custards and mayonnaises/dressings.

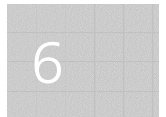
Attribute	Product group	Definition
Odor		
Vanilla	Custard	Intensity of the odor of vanilla.
Synthetic/sickly	Custard	Artificial, sickly odor of custard.
Egg	Mayo	Intensity of the odor of boiled egg yolk.
Sweet	Mayo	Intensity of a sweet odor.
Flavor/taste		
Bitter/chemical	Custard	Degree in which the taste of a product is bitter.
Vanilla	Custard	Intensity of vanilla flavor.
Oily/fatty	Mayo	Degree in which the taste of a product is oily/fatty
Sour	Mayo	Intensity of the sour flavor (as in vinegar, lemon, or dairy products).
Mouth feel		
Temperature (custard)	Custard & Mayo	Foods may elicit different temperature sensations while presented at the same physical temperature. Sensation is sensed during first contact between food and tongue
Cold (mayo) (warmer-colder)		
Thickness	Custard & Mayo	Represents the thickness of the food in the mouth after the food is compressed via up- and down motions of tongue against palate. For less viscous products like dressings, information is also obtained from the rate of spreading of the product throughout the mouth.
Heterogeneity	Mayo	A product is simultaneously perceived as thick and thin. Also referred to as "cloudy".
Melting (slow - quick)	Custard	Food becomes thin in the mouth and spread throughout the mouth at different rates.
Creamy/soft	Custard & Mayo	Range of sensation typically associated with fat content such as full and sweet taste, compact, smooth, not rough, not dry, with a velvety (not oily) coating. Food disintegrates at moderate rate.
Smooth	Mayo	Sensation: property of moving food bolus. Examples of smooth products are jelly. An example of a grainy product is semolina. Mechanism: sensation is perceived by moving the food with the tongue along the palate or teeth.
Prickling	Mayo	A prickling, stinging, biting sensation that one wants to extinct, typically perceived at the top and side surfaces of the tongue.
After feel		
Coating	Mayo	The residual layer of food after swallowing or expectoration that produces a velvety sensation.
Sticky	Mayo	Foods leave a sticky feeling in the whole mouth making it difficult to remove.
Fat	Custard & Mayo	Food leaves a fatty/oily feeling in mouth after swallowing.
Astringent	Custard & Mayo	Food leaves an astringent taste and feeling in the mouth, typically caused by products like wine, nuts and spinach.

Anchors, where not indicated: very little – very much. The order of attributes within categories and product groups is based on the temporal order in which they are perceived during mastication, as indicated by the QDA panel.

Table 2: Modified behaviors performed for five seconds with food in the mouth

Modified behavior	Definition
STILL	Stimulus is placed on the anterior 1/3 of the tongue, held for 5 seconds and then swallowed
UP	Stimulus is placed on the anterior 1/3 of the tongue, which is then raised to contact the hard palate, held for 5 seconds and food then swallowed
UP & DOWN	Stimulus is placed on the anterior 1/3 of the tongue, which is then raised to contact the hard palate, lowered, and food swallowed after 5 seconds.
SUCK	Stimulus is placed on the anterior 1/3 of the tongue, the posterior 2/3 of the tongue then raised and lowered 10 times in 5 seconds prior to swallowing
SMEAR	Stimulus is placed on the anterior 1/3 of the tongue, the tip of the tongue is moved in a figure of 8 pattern against the hard palate 10 times in 5 seconds, then food is swallowed
NORMAL	Assessment is made 5 s after ingestion, then food swallowed in the subject's normal style

Mouth-feel attributes were rated immediately following these five seconds, after which the product was swallowed and after-feel attributes rated. Foods were delivered to the mouth by inverting a spoon onto the tongue for all conditions other than normal.



RESULTS

Averaged ratings are shown per product, attribute, and modified behavior in Table 3. Many attributes, in particular those referring to mouth-feel and flavor, showed a gradual increase in rated intensity with increasing complexity of the modified behavior. The ratings given when subjects were not asked to modify normal behavior were virtually always maximal.

As indicated in Table 3, behavior modification did not affect odor perception, which was expected since odor perception preceded ingestion. Secondly, behavior modification affected most of the mouth-feel attributes and all of the flavor attributes. Finally, behavior modification affected some after-feel sensations, particularly in the case of custards. Typically, the effects were small, as demonstrated by the relatively few significant differences (in bold).

A cluster analyses based on all attributes and products returned two clusters of modified behaviors based on sensory effects. One cluster consisted mainly of the still condition and the compression-related conditions (tongue up, and tongue up and down), whereas the other cluster consisted of the compression plus shear-related conditions (suck, smear, and normal conditions).

Table 3. Average attribute ratings for mayonnaises/dressings and custard desserts by modified behavior.

Chron.Order	Dressing							Mayonnaise							Product	Beh.Modif.	Interaction
	Still	Up	Up & Down	Suck	Smear	Normal	Still	Up	Up & Down	Suck	Smear	Normal					
A																	
1	Eqq O	37	45	40	43	43	44	46	48	44	48	50	45	ns	ns	ns	
2	Sweet O	36	40	35	38	39	44	41	40	40	38	40	39	ns	ns	ns	
	Avg. Odors	37	43	38	41	41	44	44	44	42	43	45	43				
1	Cold M	31	31	33	37	36	36	39	40	42	47	46	45	**	**	ns	
2	Thick M	34	43	49	50	50	57	40	47	51	50	45	52	**	ns	**	
3	Hetero M	21	25	27	25	32	37	25	25	26	31	33	36	*	ns	ns	
4	Cream M	23	30	30	47	45	48	28	30	35	45	46	48	**	ns	ns	
5	Smooth M	23	28	30	40	42	46	25	34	39	46	49	56	**	**	ns	
6	Prickly M	33	34	34	32	36	40	35	40	40	40	44	43	ns	**	ns	
	Avg. Mo-feel	28	32	34	38	40	44	32	36	39	43	44	47				
1	Oily Fl	26	30	32	40	43	46	29	35	41	41	50	50	**	**	ns	
2	Sour Fl	31	31	37	32	36	41	33	40	41	42	47	40	*	**	ns	
	Avg. Flavor	29	31	35	36	40	43	31	38	41	41	49	45				
1	Coating A	41	45	40	43	45	45	42	43	46	46	47	49	ns	ns	ns	
2	Sticky A	30	33	32	36	35	36	33	35	36	34	37	38	ns	ns	ns	
3	Fatty A	36	40	37	37	41	43	45	45	46	47	50	54	ns	**	ns	
4	Astring A	30	32	33	31	31	32	28	29	28	29	31	31	ns	ns	ns	
	Avg. Af-feel	34	38	36	37	38	39	37	38	39	39	41	43				

To further quantify the effects of behavior modifications, the percentage difference between

B	Low fat custard							Full fat custard							Product	Beh.Modif.	Interaction
	Still	Up	Up & Down	Suck	Smear	Normal	Still	Up	Up & Down	Suck	Smear	Normal					
1	Van O	10	10	9	11	11	11	10	12	10	13	11	10	ns	ns	ns	
2	Synth O	11	11	10	14	14	11	9	11	10	10	10	10	ns	ns	ns	
	Avg. Odors	11	11	10	13	13	11	10	12	10	12	11	10				
1	Temp M	27	36	35	35	38	37	28	34	33	37	39	39	**	ns	ns	
2	Thick M	35	36	43	44	42	47	29	29	31	35	32	35	ns	**	ns	
3	Melt M	19	29	38	41	46	48	30	41	43	51	57	55	**	**	ns	
4	Cream M	25	23	26	28	33	31	32	33	39	50	53	53	**	**	**	
	Avg. Mo-Feel	26	31	35	37	40	41	29	34	37	43	45	46				
1	Bitt/ChFl	22	29	31	41	46	50	20	23	23	28	31	27	**	*	**	
2	Van Fl	22	25	30	28	29	31	28	33	35	51	50	52	**	**	*	
	Avg. Flavor	22	27	30	35	37	41	24	28	29	39	41	39				
1	Fatty A	29	33	30	31	31	35	42	46	43	46	46	45	ns	**	ns	
2	Astring A	42	45	45	46	49	48	27	27	34	26	34	31	*	**	ns	
	Avg. Af-feel	36	39	38	39	40	42	35	37	39	36	40	38				

Ratings in bold are significantly different from the corresponding ratings in the normal condition. Levels of significance of main effects of product (Product), modification condition (Beh.Modif.) and their interaction are indicated in the three right-hand columns. * $p \leq 0.05$, ** $p \leq 0.01$. O, odor; M, mouth-feel; A after-feel; Fl, flavor.

the ratings of the least complicated condition (still condition) and the normal condition were calculated by product group across fat levels (Table 4).

Table 4: Percentage difference between ratings in the normal and still conditions.

Mayonnaises	Custards						
	Low fat	High fat	All				
Egg O	16	-2	6	Vanilla O	13	-4	4
Sweet O	21	-5	7	Synt/sickly O	1	13	7
Avg. Odor	19	-3	7	Avg. Odor	7	5	5
Oily/fatty Fl	78	73	76	Bitter/chem. Fl	127	32	81
Sour Fl	34	23	29	Vanilla Fl	42	87	67
Avg. Flavor	56	48	52	Avg. Flavor	84	60	74
Cold M	15	16	15	Temperature M	35	41	38
Thick M	68	31	48	Thick M	36	23	30
Heterogeneous M	72	40	55	Melting M	159	84	113
Creamy/soft M	107	68	86	Creamy/soft M	28	69	51
Smooth M	103	122	113				
Prickling M	20	23	21				
Avg. Mouth-feel	64	50	56	Avg. Mouth-feel	64	54	58
Coating A	9	18	14				
Sticky A	20	13	16				
Fatty A	20	19	19	Fatty A	20	9	13
Astringent A	5	10	7	Astringent A	15	18	16
Avg. After-feel	13	15	14	Avg. After-feel	18	13	15

Results are given per attribute and averaged across attributes per category (O=odor, Fl=flavor, M=mouth-feel, A=after-feel).

The averaged results in Table 4 indicate that flavor and mouth-feel attributes were affected to a similar degree. Secondly, per attribute category, differences between still and normal conditions are very similar for mayonnaises and custards. Thirdly, increasingly complex oral behaviors seemed to affect mouth-feel for mayonnaises more than mouth-feel for custards, at least for those attributes that were used for both types of products. On average, thickness ratings for mayonnaises and custards increase with 48% and 30%, respectively between still to normal behaviors. Similarly, creaminess mouthfeel ratings for mayon-naises and custards increase with 86% and 51%, respectively (see also Fig 1).



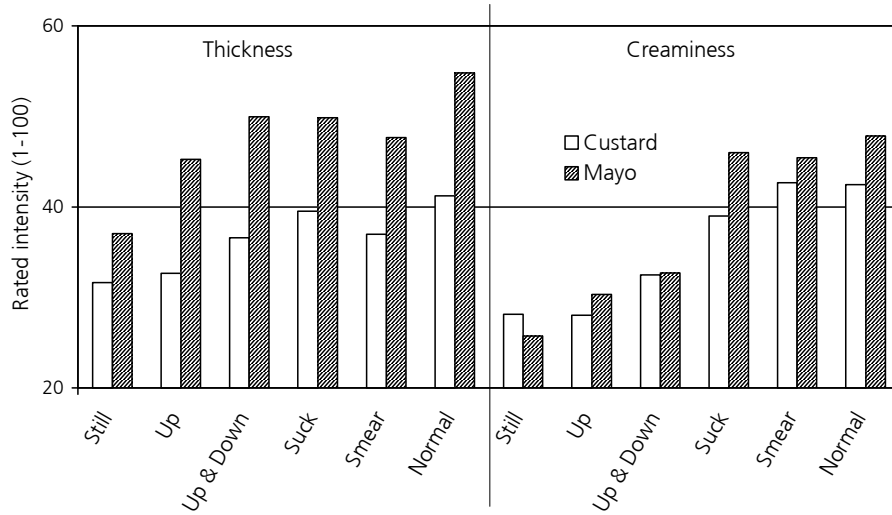


Fig 1. Ratings of perceived thickness and creaminess for mayonnaises and custards per modified behavior

Fourthly, mouth- and after-feel effects indicate that low fat products may be more affected by behavior modifications than high fat products. Finally, for mouth-feel attributes, especially in the case of mayonnaises, attributes that showed the smallest effects of behavior modification were typically the ones capable of being rated soonest after ingestion. One exception was prickling, which showed a small effect of behavior modification but was rated last by the QDA panel (Fig. 2).

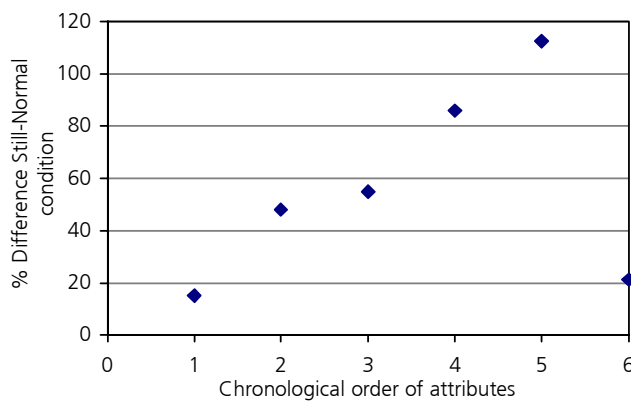


Fig 2. Percentage change (relative to the still condition) of attributes relative to the chronological order in which they are perceived

DISCUSSION

This study investigated the effect of specific masticatory movements on flavor, mouth-feel and after-feel sensations for high and low fat versions of two semi-solid foods, mayonnaises and vanilla custard desserts. The results indicated a gradual increase in the intensity of sensations, in particular those of mouth-feel and flavor, with the complexity of inter-oral movement. Typically, normal oral processing resulted in the most intense sensations. The classification of normal oral processing behavior of consumers for semi-solids (see introduction) indicates that these behaviors vary widely between consumers but that they share a high degree of complexity (15). The present results indicate that the degree of complexity observed in the oral processing behavior of consumers indeed results in the most intense sensations. This suggests that consumers aim to maximize their food sensations, at least for the foods investigated here. It is not clear whether other, less desirable foods would result in oral processing behavior aimed to minimize food sensations.

Results also indicate that the sensation of virtually all flavor and mouth-feel attributes requires at least some tongue movement. These manipulations mix food with saliva and thereby enhance mechanical and chemical breakdown, and position the food relative to the sense organs. The effects of tongue movements on flavor sensation can be explained by increased flavor release during food breakdown and by the redistribution of the food over a larger area of the tongue. Since flavor is a combination of retro-nasal olfaction and taste, movement of the tongue pumps volatile compounds into the nose thereby enhancing flavors (16)

The general effect of tongue movements on mouth-feel attributes defined as the percentage difference in ratings between the least complicated and normal conditions, was related to specific attributes. The attributes least affected by tongue movements were typically those rated soonest after ingestion (see Figure 2), in other words those that require no or only a few tongue movements to assess. A noticeable exception is prickling, which is perceived relatively late in the oral processing cycle but requires very little tongue movement (Figure 2), possibly reflecting the time needed to activate the oral trigeminal system (17;18). The specific tongue movements required by each of the other mouth-feel attributes provide information on the underlying mechanisms. Although this study did not include oral physiological measurements to shed light on the exact nature of these mechanisms, results suggest that:

Temperature (or coldness) perception requires little or no tongue movement and relatively short residence times in the mouth, and this is one of the first attributes that can be rated. The sensation may arise during the initial moments of contact between the product and the oral tissue, when the temperature difference is at its maximum (19). It can be hypothesized that to protect the individual, the sensation must arise immediately to prevent possible tissue damage. At cold temperatures, the tip of the tongue responds more strongly to temperature than other parts of the mouth (20). This finding is confirmed by another study performed in this laboratory, where palatal coverage failed to affect the perceived temperature of stimuli.



Prickling was the only other mouth-feel attribute that required little or no tongue movement to be perceived at full strength.

It is not possible to categorize mouth-feel attributes by the type of movement required for sensation (e.g., attributes related to compression-only movements versus those related to compression plus shear movements). The exact nature of these movements does not appear to be related to specific mouth-feel attributes but rather to product group and even to individual products. For example, thickness ratings for mayonnaises showed a considerably larger effect of tongue movements than corresponding ratings for custards, and low fat mayonnaises showed a larger effect than high fat ones, which only increased with the tongue-up movement and remained virtually stable thereafter (see Table 4).

These results suggest that there may be multiple ways to establish ratings for mouth-feel attributes. This was also suggested by the panelists themselves who define for example thickness by the food's compression behavior as well as by its spreading behavior, depending on its viscosity (Table 1).

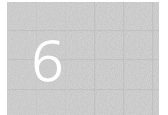
In summary, an individual's normal oral processing behavior typically resulted in the most intense sensations of flavor and mouth-feel. Oral residence time for all mouth-feel attributes, except prickling, was determined by the time required for tongue movements. The exact tongue movements required for sensations appeared to be determined by product groups and individual products, rather than by specific mouth-feel attributes.

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CHAPTER 7

THE EFFECT OF ORAL TEMPERATURE ON THE
TEMPERATURE PERCEPTION OF LIQUIDS
AND SEMI-SOLIDS IN THE MOUTH

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ABSTRACT

This work examined the influence of oral temperature on oral perception of temperature in liquids and semi-solids. A panel of 20 adults assessed the temperature of water, custard dessert and mayonnaise. Oral temperatures were manipulated by 5 second mouth rinses of 10°C, 35°C and 55°C performed prior to assessments, which resulted in oral temperatures of 27°C, 35°C and 43° C, respectively. The products were evaluated at 10°C, 22°C and 35°C. Results show that subjects were able to differentiate between the product temperatures. A large effect of type of product was seen on perceived temperature, where water was over-all perceived as significantly colder than custard dessert and mayonnaise. The range of perceived thermal ratings was widest for custard dessert, followed by water and mayonnaise. This might be due to differences in composition and structure of the products. Even though oral temperature was varied considerably in the present study, this did not exert large effects on perceived temperature.

INTRODUCTION

Humans have a strong preference for the temperature of the products they consume. The preferred intake temperature of water was reported to be 15°C irrespective of hydration status of subjects (1). Even though very cold water (5°C) was reported to be the most pleasurable, maximal intake was observed at 15°C. Different products exhibit diverse preferred intake temperatures; ice cream is considered most pleasant when eaten cold and a hamburger tastes the best when it is warm. Other products are consumed at different temperatures, depending on the context and culture. For example, in most countries mayonnaise is eaten cold in salads etc, but can also accompany hot foods such as french-fries. The development of these preferences reflects the presence of a sensitive intra-oral system for perceiving temperature. Temperature sensation on the skin has been shown to differ depending on ambient temperature, with cool environments resulting in less strong sensations than neutral environments (2). If perceived temperature is a measurement of the difference in temperature between oral mucosa and stimuli, it would be dependent on oral temperature. Hence, would a product taken from the fridge elicit the same temperature sensation if ingested on a cold winter day as in a hot desert?

Several authors investigated some of the qualitative aspects of temperature perception (3), including warm and cold receptors (4), comparisons between different loci (5;6), effects on liking (1;7;8), taste (9;10), irritation (11;12), age (13), and spatial summation (14;15). Skin temperature is perceived by specific cold and warm receptors (16). These receptors are slowly adapting units that exhibit a steady-state discharge at constant skin temperature, a dynamic response to temperature changes and insensitivity to mechanical stimuli (17;18). In addition to the specific thermal receptors, other types of receptors, such as mechanoreceptors and multimodal fibers can respond to thermal stimuli. Even though cold receptors are most often innervated by myelinated A δ afferents, and warm receptors are innervated by C-fibers, this separation is not absolute (19;20). There is only little literature on oral thermoreceptors, but it is expected that these are similar to cutaneous thermoreceptors elsewhere on the body.

In this study, oral temperature was manipulated by rinsing the mouth with water of various temperatures. To investigate the perception of temperature water, custard dessert (a low-fat dairy product) and mayonnaise (a high-fat oil-in-water emulsion product) at three different temperatures were used as stimuli. These products varied largely not only in fat percentage, but also in viscosity and other physical characteristics. An unpublished study in our laboratory indicated that perceived temperature of semi-solids correlated with fat content. A higher fat content resulted in higher temperature ratings than products with lower fat content. Thus, the possible effects of different product characteristics on thermal perception could be investigated.

This study sought to examine the mechanisms underlying temperature perception by investigating the effects of oral temperature on the perception of product temperature. We



were interested in whether temperature perception is solely dependent on the physical temperature and characteristics of the food product, or on the difference between the consumed product and oral mucosa. The extent to which oral and product temperatures interact to produce a sensation of temperature was also investigated.

MATERIAL AND METHOD

Subjects

Twenty healthy Caucasian volunteers (6 male and 14 female, 18-35 yr) without any neurological disorders were selected for this study on the basis of a well functioning smell and taste perception. The subjects gave informed consent and were compensated for their participation. Each subject was always tested at the same time of the day.

Stimuli

Pure tap water and two types of commercially available semi-solids were chosen for their widely different fat content. Boerenland vanilla custard dessert (4.3% fat, Campina BV, Deventer, the Netherlands) and Calvé mayonnaise (72% fat, Unilever Best Foods BV, Vlaardingen, the Netherlands) were selected. Custard dessert and mayonnaise also differ in other physical properties, such as viscosity and thermal properties. Exact values for thermal conductivity (λ) and heat capacity (C_p) are not known for custard dessert and mayonnaise, but can be approximated to the values for water ($\lambda = 0.67\text{W/m}\cdot\text{K}$, $C_p = 4.19\text{kJ/kg}\cdot\text{K}$) and oil ($\lambda = 0.14\text{W/m}\cdot\text{K}$, $C_p = 2.12\text{kJ/kg}\cdot\text{K}$), respectively, when product ingredients are considered.

Procedure

Rinse water was served at three temperatures: 10°C (refrigerator), 35°C and 55°C (both heated and kept in a controlled water bath) prior to assessment.

Water, custard dessert and mayonnaise were served at three different temperatures: 10°C (refrigerator), 22°C (room temperature) and 35°C (climate cupboard). The products were put in 70 ml polystyrene cups containing 20 ml and the cups were placed in their appropriate locations for three hours prior to serving to allow the product stimuli to reach the desired temperature.

A cup of tempered rinse water was always administered to the subjects together with a climatised product stimulus. Each subject received all combinations of the three rinse water temperatures (10°C, 35°C and 55°C), three product stimuli (water, custard dessert and mayonnaise), and three product temperatures (10°C, 22°C and 35°C) in triplicate. In order to avoid adaptation and saturation effects, the samples were administered randomly, and the 81 samples were divided over three weekly one-and-a-half hour sessions. During these sessions, a 15-minute pause was included to avoid fatigue. The interval between the samples was 2.5 min.

Sensory procedure

The subjects were seated in sensory booths with appropriate and controlled ventilation and lighting. The subjects swirled the rinse water around in the mouth for five seconds and then expectorated it. Immediately after rinsing, subjects took one spoonful of product and rated the temperature by magnitude estimation on a 100-point visual analog scale (VAS) ranging from cold to hot.

Intra-oral temperature measurements

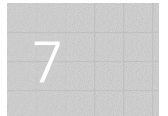
The intra-oral changes in temperature after mouth rinses were analyzed by an infra-red thermometer (RayMX4G, Raytek GmbH, Berlin, Germany). Subjects followed the same rinsing regime as previously described in the procedure section. Before and after a mouth rinse lasting for five seconds with water at 10°C, 35°C and 55°C, the temperature of the oral mucosa was measured.

Physical measurements

In order to investigate the effect of temperature on physical properties, the viscosities of the products were measured at the three product temperatures. The measurements were performed with a rheometer (Physica MCR 300, Paar Physica GmbH, Stuttgart, Germany), by placing the samples in a gap of 1 mm between two horizontal plates, 40 mm in diameter. Water resistant sandpaper was glued to the surface of both plates in order to prevent slippage. The torque was measured during rotation of the plates at a constant shear rate of 10s⁻¹. Each measurement lasted 64.8 s and composed of 200 measuring points. The viscosities were calculated from the 2 s data points.

Data analysis

Subject ratings were gathered and analyzed by FIZZ software (1998, Biosystèmes, Couternon, France). Repeated-measures ANOVAs (SPSS 9.0, SPSS Inc., Chicago, IL) with Greenhouse-Geisser as correction factor, were carried out on data averaged across three replicates. Product temperature, oral temperature and type of product were included as within-subject factors. Gender effect was analyzed and the intra-class correlation coefficient was calculated. $p < 0.05$ was considered significant.



RESULTS

Following mouth rinses with water at 10°C, 35°C and 55°C, the resulting mean intra-oral temperatures measured by the infra-red thermometer were 27°C, 35°C and 43°C, respectively. Average temperature ratings are shown per product, product temperature and oral temperature in Table 1, and the results of the data analysis are shown in Table 2.

Table 1. The average ratings (1-100) and SD of perceived temperature for three products at three product temperatures and three oral temperatures

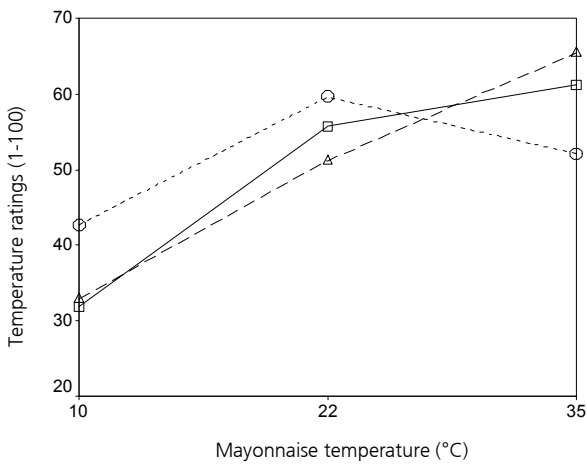
Product temperature (°C)	Oral temperature (°C)	Water	Mayonnaise	Custard dessert
10	27	26.9 (9.2)	33.0 (14.0)	23.7 (9.8)
	35	27.6 (10.6)	31.8 (10.5)	25.7 (8.1)
	43	28.9 (15.2)	42.7 (15.8)	23.2 (7.5)
22	27	43.7 (12.6)	51.2 (14.8)	53.0 (13.0)
	35	42.3 (13.1)	55.7 (11.2)	53.0 (12.0)
	43	39.7 (14.6)	59.6 (14.0)	49.0 (13.5)
35	27	60.4 (12.0)	65.4 (12.1)	70.0 (11.9)
	35	56.0 (14.0)	61.2 (11.8)	65.9 (9.2)
	43	54.5 (13.1)	52.1 (23.1)	66.6 (8.7)

Table 2. F- and P-values of the main and interaction effects (by ANOVA)

Main effects	F	P
T _{product}	756	0.000
T _{oral}	1.16	0.314
Product	47.9	0.000
Interactions		
T _{product} × T _{oral}	8.4	0.000
T _{product} × Product	30.4	0.000
T _{oral} × Product	1.6	0.167
T _{product} × T _{oral} × Product	4.6	0.000
Gender effect	0.316	0.376

No effects of gender could be seen, and the intra-class correlation coefficient was 0.2, suggesting that that the inter-subject variability was significantly higher than the intra-subject variability. Product temperature had a strong effect on perceived temperature ($p < 0.001$), with higher product temperatures resulting in increasing temperature ratings. Oral temperature failed to show a significant over-all effect ($p = 0.31$).

However, oral temperature showed opposite effects on cold and hot mayonnaise (interaction, $p < 0.001$) (Fig 1). While this effect was strong for mayonnaise, it was not observed for water and custard dessert.



The cold mayonnaise (10°C) was perceived as colder ($p < 0.001$) when taken into a cold or warm mouth (oral temperatures 27°C and 35°C, respectively) than when taken into a hot mouth (oral temperature 43°C). The warm mayonnaise (35°C), on the other hand, was perceived as warmer ($p < 0.001$) when taken into a cold mouth (oral temperature 27°C) than when taken into a warm or hot mouth (oral temperatures 35°C and 43°C, respectively).

Fig 1. The interaction effect of oral and product temperature of mayonnaise (oral temperature: squares, 27°C; circles, 35°C; crosses 43°C, respectively)



The results showed a significant over-all product effect ($p < 0.001$) on the perceived temperature, where water was on average being perceived as coldest, followed by custard dessert and mayonnaise. There were significant interactions between the type of product and the product temperature ($p < 0.001$) (Fig 2). Differences in the temperature of the mayonnaise were relatively poorly detected (range of 23 VAS-units) when averaged over oral temperatures. In contrast, temperature differences of custard dessert were even better detected (range of 41 VAS-units), than those of water (32 VAS-units). This was evident from the high scores recorded at medium and warm product temperatures.

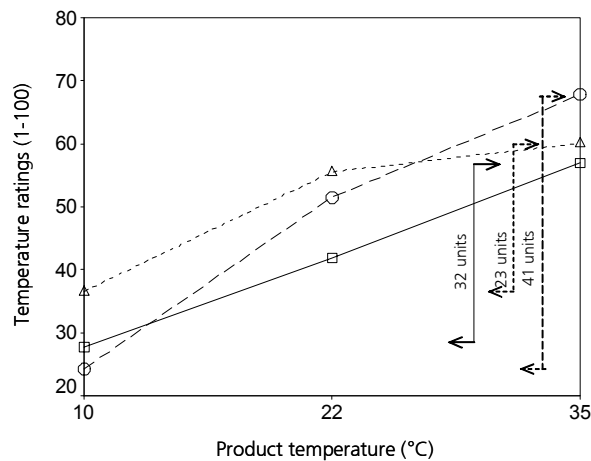


Fig 2. Interaction between product (squares, water; triangles, mayonnaise; circles, custard dessert) and product temperature on the mean temperature ratings across all oral temperatures.

Physical measurements

The viscosities of custard dessert and mayonnaise were influenced by temperature. The trend was that the higher the temperature, the lower the viscosity. A decrease in viscosity of 53 % and 30 % for custard dessert and mayonnaise respectively was seen when product temperature was varied from 10°C to 35°C.

DISCUSSION

In this study we investigated the effect of oral and product temperature on the perceived temperature of liquids and semi-solids. Three widely diverse products (water, custard dessert and mayonnaise) were included in this study, since different physical characteristics of the product could be important in temperature perception.

The fact that the subjects rated cold products colder than warm products is evidence for an existing and working oral thermal sense. Mouth rinse of different temperatures was shown to be capable of changing the temperature of the intra-oral mucosa. The maximum temperature difference between mucosa and product was 33°C; this was the case with a heated mouth and a cold product. These findings are in accordance with previous research where the *in vivo* temperature of teeth during meals was investigated (21;22). It was reported that the maximum difference in temperature was 29°C and 28°C, respectively. Even though oral temperature varied considerably in the present study, this failed to exert large effects on perceived temperature. An explanation for this could be found in the experience during the respiration cycle. During inhalation, a large amount of air at ambient temperature is taken in, either through the mouth or through the nose. The air that is exhaled, is heated in the lungs and is at body temperature. During cold days these temperature changes can be considerable. The mucosa of the mouth is thus used to a large variation in temperature, and could disregard this information either at receptor- or at higher integration levels.

However, product temperature was not judged as an absolute entity by the panelists. This becomes clear when comparing the temperature ratings of mayonnaise at the three oral temperatures. Temperature was rated in comparison to the condition just prior to intake, i.e. as a difference between oral mucosa temperature and product temperature. Temperature was rated highest when warm mayonnaise was taken into a cold mouth, such as after a rinse with cold water. In addition, differences in temperatures of the mayonnaise were less perceptible when the mouth was warmer than resting baseline (35°C). Since the different product temperatures could be separated clearly by the subjects, we can look at the separate product temperatures and focus on heat transfer as a possible mechanism. At a product temperature of 10°C the mayonnaise was perceived as being coldest when taken into a cold or warm mouth. This may be accredited to the heat transfer, where a cold product draws heat from the already cold or neutral mouth, resulting in a cold sensation. In contrast, as the cold mayonnaise was taken into a hot mouth the product was rapidly heated, and while the mouth was only cooled down to neutral temperatures, the mayonnaise was perceived as warmer. When examining the ratings for the warm mayonnaise (35°C), we see the following: when taken into a cold mouth

the mayonnaise was perceived as the warmest, due to the heat transfer from the warm product to the cold mouth. It is worth noting that this is the only combination in this study where the temperature of the product was actually higher than the temperature of the mouth. Accordingly, a colder sensation was experienced when heat was transferred from the mouth to the product, such as with a hot mouth.

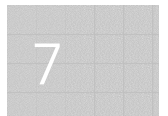
There was a large difference in perceived temperature between the products, where water was perceived as significantly colder than the other stimuli. When looking at the ratings for the separate temperatures, however, we see that the range of the mean ratings between cold and warm products over all oral temperatures differ between the products, with mayonnaise showing the narrowest and custard dessert the widest range (Fig 2). One possible explanation for this could be found in the spreadability of the products, where mayonnaise is more viscous and prone to remain as a large droplet in the mouth compared with water and custard dessert. Mayonnaise will initially spread less in the mouth and possibly activate a smaller amount of sensors, resulting in less strong sensations. An alternative explanation could be found in the thermal nature of the products, where due to the low thermal conduction coefficient of oil in comparison to water, a high fat product insulates better, being perceived as less cold at low temperatures and less warm at high temperatures than water. The reason why custard dessert exhibited such a large range, especially the high ratings at high temperature, is still unanswered. The rapid change in viscosity of custard dessert on heating might be of importance. Next to purely physical conditions, cognitive aspects may also exert an influence. Custard dessert, for example, is always eaten cold in the Dutch community. Accordingly, the thermal sensations of warm custard dessert might be enhanced, resulting in higher temperature ratings.

Another study performed in our laboratory has shown that a substance only needs to be in the mouth for a short time to rate the perceived temperature (de Wijk, personal communication). As temperature was one of the first attributes to be rated during these psychophysical measurements, coldness may be a sensation arising during the initial moments of contact between the product and the oral tissue, when the temperature difference is at its maximum (4). It can be hypothesized that for means of protection the sensation has to arise immediately in order to prevent any possible damage to the tissues.

The present data shows that the oral perception of temperature is affected by the type and characteristics of the product ingested, and that the temperature of the oral mucosa is of minor importance.

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CHAPTER 8

THE EFFECT OF ORAL AND PRODUCT
TEMPERATURE ON THE PERCEPTION OF FLAVOR
AND TEXTURE ATTRIBUTES OF SEMI-SOLIDS

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ABSTRACT

This study examined the effect of oral and product temperature on the perception of texture and flavor attributes. A trained panel assessed 21 texture and flavor attributes in one high-fat and one low-fat product of two semi-solids: custard dessert and mayonnaise. The products were evaluated at 10°C, 22°C or 35°C in combination with oral temperatures of 27°C, 35°C and 43°C.

Results showed that modulation of product and oral temperature had significant effects on a number of attributes. Flavor intensities, melting mouth feel, and fat after feel increased, while subjective thickness decreased with increasing product temperature. Neither product- nor oral temperature had an effect on over-all creaminess. Oral temperature affected a number of mouth feel attributes: melting, heterogeneous and smooth. Furthermore, large differences existed in ratings between the high- and low-fat products of custard and mayonnaise, and they were more prominent in mayonnaise.

We conclude that the effect of oral temperature on the perception of sensory attributes in semi-solids was small, but present, while the product temperatures influenced the ratings greatly.

INTRODUCTION

Texture of semi-solids is of importance for their acceptance. The definition of food texture as proposed by Matz (1) is: "the mingled experience deriving from the sensations of the skin in the mouth after ingestion of food or beverage, as it relates to density, viscosity, surface tension and other physical properties of the material being sampled". Hence, a change in physical property of food would influence the texture sensation. For instance, an increase in product temperature is known to change the viscosity, cause melting of fats and enhance flavor and odor release. It is not known however, what the resulting effects of changes in temperature on texture attributes in semi-solids are. The present study investigated the effects of temperature on texture attributes.

Humans have a strong preference for the temperature of the products they consume (2;3). Different products exhibit diverse preferred intake temperatures; ice cream is considered most pleasant when eaten cold and French-fries taste the best when warm. Other products are consumed at different temperatures, depending on the context and culture. For example, in most countries mayonnaise is eaten cold e.g. in salads etc, but can also accompany hot foods such as french-fries. During sensory evaluations a product is served and the attributes rated at the temperature at which the product is usually eaten. This was also done in previous studies by our team (4;5) for the semi-solids custard dessert and mayonnaise, which were served at 10°C. In those studies a sensory fingerprint was established for the products at that temperature. However, the serving temperature might influence the ratings of sensory attributes.

Temperature effects on texture perception can be mediated by physico/chemical changes in the product, or by differences at the level of the mucosa. In addition to sensory phenomena, these effects may also be perceptual. Product temperature could influence the viscosity of the product and the ratio of solid and melted fat and thereby influence the quality and the thickness of the oral coating formed. The differences in oral temperature could affect receptor response, blood flow and have a secondary effect by altering the product on contact, all of which may change the response to the stimuli. If oral temperature is important, it can be hypothesized that heating or cooling the mouth can modify sensory ratings. Influences of oral and product temperature on the perception of fat level in a liquid emulsion have been investigated (6). The oral temperatures were manipulated using water of different temperatures to rinse the mouth. They found no effects of the mouth rinse nor of the variations in stimulus temperature. Cooling of the skin has been shown to attenuate or completely turn off the burning sensation of capsaicin and other irritants (7-9), while heating tends to synergize with chemical stimulation, which heightens sensory irritation (7;10-13). Similar results are seen for roughness, where apparent roughness is shown to decline as skin temperature falls below normal, and tends to be enhanced as skin temperature rises above baseline (14). The perception of tastants can also be affected by temperature(15). NaCl sensitivity was higher at solution temperatures of 22°C and 37°C than at 0°C or 55°C (16). The perceived sweetness of



sucrose solutions of low concentrations was reported to vary directly with solution temperature (17-19), where the sweetness was greater at higher temperatures. Cooling of the tongue reduced sweetness more than did cooling the solution (20). Taste sensations have been evoked by thermal stimulation of the tongue: A cold stimulus at the anterior of the tongue can evoke sourness and/or saltiness, whereas warming can evoke sweetness (21). Hence, there is evidence that temperature affects various sensations in the mouth and on the skin, but only little is known about the effects of temperature on texture perception of semi-solids.

The aim of this study was to investigate the effects of product and oral temperature on the perception of texture and flavor attributes in semi-solids. We expected both temperatures to exert important effects on sensory perception. Accordingly, we were interested in whether sensory perception is solely dependent on the physical temperature and characteristics of the food product, or on the difference between the consumed product and oral mucosa. The extent to which oral and product temperatures interact to produce a sensation was also investigated. We examined a high- and a low-fat product of two semi-solid groups in this study, because fat is suggested to be an important component due to its possible involvement in flavor and texture sensation.

MATERIALS & METHODS

Subjects

20 healthy volunteers (6 male and 14 female, 18-35 years) without any neurological disorders were selected for this study on the basis of a well functioning smell and taste perception. All subjects had previously been screened for olfactory and taste disorders and had received extensive training in rating odor, flavor, mouth- and after feel attributes of custard desserts and mayonnaises. The subjects gave informed consent and were compensated for their participation. Each subject was always tested at the same time of the day.

Stimuli

Two types of commercially available vanilla flavored Dutch custard desserts (products thickened by starch and hydrocolloids, like carrageenan) and two types of mayonnaises (oil-in-water emulsions) were chosen for their widely different fat contents and sensory profiles. A trained descriptive sensory panel affiliated with this research group had established these profiles (4). A low-fat (0.2% fat) and a high-fat custard dessert (4.3% fat), and a low-fat (32% fat) and a high-fat (72% fat) mayonnaise were selected. The terms low- and high fat are, though widely different in the two types of product, related to the normal fat % of the specific type of product. Custard dessert and mayonnaise differ in a number of physical properties, such as viscosity and thermal properties, due to the differences in ingredients and production methods. Exact values for thermal conductivity (λ) and heat capacity (C_p) are not known for custard dessert and mayonnaise, but can be approximated to the values of the main

ingredients: water ($\lambda = 0.67 \text{ W/m} \cdot \text{K}$, $C_p = 4.19 \text{ kJ/kg} \cdot \text{K}$) and oil ($\lambda = 0.14 \text{ W/m} \cdot \text{K}$, $C_p = 2.12 \text{ kJ/kg} \cdot \text{K}$), respectively.

Procedure

Rinse water was served at three temperatures: 10°C (refrigerator), 35°C and 55°C (both heated and kept in a controlled water bath) prior to assessment.

The custard desserts and mayonnaises were served at three different temperatures: 10°C (refrigerator), 22°C (room temperature) and 35°C (climate cupboard). The products were put in 70 ml polystyrene cups containing 20 ml and the cups were placed in their appropriate locations for three hours prior to serving to allow the product stimuli to reach the desired temperature.

A cup of tempered rinse water at one of the three temperatures to rinse the mouth with was always administered to the subjects together with a climatized product stimulus. Each subject received all combinations of the three rinse water temperatures (10°C, 35°C and 55°C), two product stimuli (custard dessert and mayonnaise), two types (high-fat and low-fat) and three product temperatures (10°C, 22°C and 35°C) in triplicate. In order to avoid adaptation and saturation effects, the samples were administered randomly, and the 108 samples were divided over four one-and-a-half hour sessions. During these sessions, a 15-minute pause was included to avoid fatigue. The same group of subjects participated in a variety of sensory studies in 40 yearly sessions over a period of 3 years. The pace of sample presentation during a session, typically one sample per 3 minutes, was experienced as comfortable and subjects never indicated that they were overburdened

Attributes

The attributes used in the studies were selected as a representative sub-set from a set of attributes developed previously for vanilla custard desserts and mayonnaises by a Quantitative Descriptive Analysis (QDA) panel (4;5). The selected attributes were divided into five subsets, based on their functional character. These subsets were odors, flavors, tooth-lip feel, mouth feel, and after feel (Table 1). Tooth-lip feel is the sensation arising from rubbing the upper lip against the upper front teeth and after feel is the oral sensation remaining after swallowing. As mayonnaise seems to be a more diverse type of product, a larger number of attributes was selected for the mayonnaises and some attributes are overlapping between the custard desserts and the mayonnaises.

Sensory procedure

The subjects were seated in sensory booths with appropriate and controlled ventilation and lighting. The subjects swirled the rinse water around for 5 s in the mouth and then expectorated it. Immediately after rinsing the subjects smelled the product and rated the odor. Thereafter they took one spoonful of the product and rated the flavor and texture attributes, followed by the after feel attributes directly after swallowing. The whole assessing regime



including the rinse took approximately 30 seconds. The sensory attributes were rated on a 100-point visual analog scale (VAS).

Table 1. List of selected odor, flavor and texture attributes and their related functional character of the product groups custard dessert and mayonnaise

Custard dessert		Mayonnaise	
Attribute	Functional character	Attribute	Functional character
Vanilla (od)	odor	Egg (od)	odor
synthetic/sickly (od)	odor	Sweet (od)	odor
Vanilla (fl)	flavor	oily/fat (fl)	flavor
bitter/chemical (fl)	flavor	Sour (fl)	flavor
		Rough (tl)	tooth-lipfeel
		Slippery (tl)	tooth-lipfeel
Temperature (mo)	mouthfeel	Temperature (mo)	mouthfeel
Thickness (mo)	mouthfeel	Thickness (mo)	mouthfeel
Melting (mo)	mouthfeel	Heterogeneity (mo)	mouthfeel
Creaminess (mo)	mouthfeel	Creaminess (mo)	mouthfeel
		Smooth (mo)	mouthfeel
		Pungency (mo)	mouthfeel
		Coating (af)	afterfeel
		Stickiness (af)	afterfeel
Fat (af)	afterfeel	Fat (af)	afterfeel
Astringent (af)	afterfeel	Astringent (af)	afterfeel

Intra-oral temperature measurements

Intra-oral changes in temperature after mouth rinses and during the study were analyzed by an infra-red thermometer (RayMX4G, Raytek GmbH, Berlin, Germany). The subjects followed the same rinsing regime as previously described in the procedure section. After a mouth rinse lasting for 5 s with water at 10°C, 35°C and 55°C, the temperature of the oral mucosa was measured.

Physical measurements

In order to investigate the effect of temperature on physical properties, the viscosities of the products were measured at the three product temperatures. Measurements were performed with a rheometer (Physica MCR 300, Paar Physica GmbH, Stuttgart, Germany), by placing the samples in a gap of 1 mm between two horizontal plates, 40 mm in diameter. Water resistant sandpaper was glued to the surface of both plates in order to prevent slippage. The torque was

measured during rotation of the plates at a constant shear rate of 10s^{-1} . Each measurement lasted 64.8 s and comprised of 200 measuring points. Viscosities were calculated from the data points obtained after 2 s.

Data analysis

Subject ratings were gathered and analyzed by FIZZ software (1998, Biosystèmes, Couternon, France). Repeated-measures ANOVAs (SPSS 9.0, SPSS Inc., Chicago, IL) with Greenhouse-Geisser as correction factor, were carried out on data averaged across three replicates. Oral temperature, product temperature, and type of product were included as within-subject factors. $p < 0.05$ was considered significant.

RESULTS

Following mouth rinses with water at 10°C , 35°C and 55°C , the resulting mean intra-oral temperatures measured by the infrared thermometer were 27°C , 35°C and 43°C , respectively. After the rinse, the oral temperature gradually changed and oral temperature was completely back to baseline (35°C) after approximately 2 minutes. The baseline temperature remained constant throughout the study.

The mean ratings of custard desserts and mayonnaises are shown for oral temperature, product temperature and per product in Table 2. Table 3 shows the significance of the main effects and interactions.

Custard dessert

The modulation of product and oral temperature had significant effects on a number of attributes in the custard desserts. A higher product temperature resulted in significantly higher ratings of vanilla and synthetic odors, bitter/chemical flavor, temperature and melting mouth feel, and fat after feel ratings, and lower ratings for thickness. Higher oral temperatures led to a significant increase in melting mouth feel ratings.

The high- and low-fat products were perceived differently (Fig 1a), where the high-fat product was rated as having more vanilla and less bitter/chemical flavor, being more creamy, and having more fatty and less astringent after feel than the low-fat custard.

Significant interactions between type of product and product temperature were detected. A higher product temperature enhanced creamy mouth feel and fatty after feel ratings for the low-fat custard. The opposite was true for the high-fat custard dessert, where the perception of creaminess was seen to decrease with increasing product temperature, while the ratings for fatty after feel were practically unchanged.



Table 2. Average ratings for all the odor, flavor and texture attributes at product temperatures 10, 22 and 35 °C and oral temperatures 27, 35 and 43 °C

Product	T Product	T Oral	egg-od	sweet-od	van-od	synt-od	oily-fl	sour-fl	bitt-fl	van-fl	rough-tl	slippery-tl	temp-mo	thick-mo	melting-mo	heterogeneous-mo	creamy-mo	smooth-mo	prickling-mo	sticky-mo	coating-af	fat-af	astringent-af	
Low-fat Custard	27	27			32	38			56	28			26	56	48		31					35	46	
	10	35			28	39			56	30			25	57	50		34					37	48	
		43			31	37			56	29			24	55	48		33					38	46	
		27			31	45			57	32			57	47	51		36					43	47	
	22	35			29	44			59	30			52	48	52		35					43	50	
		43			30	44			57	30			51	44	56		35					39	49	
		27			34	47			58	32			71	33	58		38					45	46	
		35	35		37	48			59	32			64	33	63		39					46	47	
		43			35	46			59	32			62	33	62		41					41	46	
		27			37	38			37	47			24	58	43		62					57	34	
High-fat Custard	10	35			38	39			37	45			26	52	47		64					58	35	
		43			40	40			37	46			23	53	49		63					55	37	
		27			41	42			45	44			53	46	51		62					59	39	
	22	35			42	40			44	44			53	46	51		63					59	39	
		43			42	43			44	43			49	46	53		63					58	40	
		27			42	45			44	45			70	39	54		61					58	40	
		35	35		40	45			46	45			66	38	55		61					60	39	
		43			41	43			46	44			67	37	58		61					58	40	
	Low-fat Mayo		27	46	48			50	48			48	43	24	63		33	57	49	42	39	44	42	43
		10	35	45	46			50	50			48	41	28	65		33	55	44	43	44	47	44	45
		43	46	45			50	48			48	40	41	63		34	55	48	44	39	47	41	41	
		27	48	49			50	48			52	40	44	60		36	56	49	43	40	51	42	41	
22		35	51	46			50	45			53	40	47	59		35	56	45	43	43	49	44	42	
		43	51	46			53	45			45	42	45	60		35	55	51	42	39	48	45	37	
		27	53	48			51	49			47	45	67	56		40	57	49	42	41	49	46	43	
		35	35	53	49			54	48			50	43	63	56		35	57	48	43	44	46	46	41
		43	52	43			52	48			50	41	53	59		36	60	50	46	41	49	45	38	
		27	52	42			56	54			41	52	33	59		39	57	54	49	40	48	50	35	
High-fat Mayo	10	35	52	43			57	56			42	48	32	57		35	59	52	51	39	50	50	35	
		43	52	38			56	54			43	46	43	57		39	58	52	51	40	48	49	36	
		27	49	37			58	56			42	50	51	52		35	58	58	51	42	46	48	35	
	22	35	51	41			59	53			40	47	56	56		33	60	54	49	41	50	49	35	
		43	56	38			60	55			40	49	60	54		33	60	56	49	40	47	51	34	
		27	48	39			58	55			41	49	65	47		39	59	60	49	37	49	50	30	
		35	35	55	39			62	56			36	50	61	49		39	59	58	46	40	45	50	30
		43	44	40			57	55			35	49	52	52		38	55	56	50	38	49	48	33	

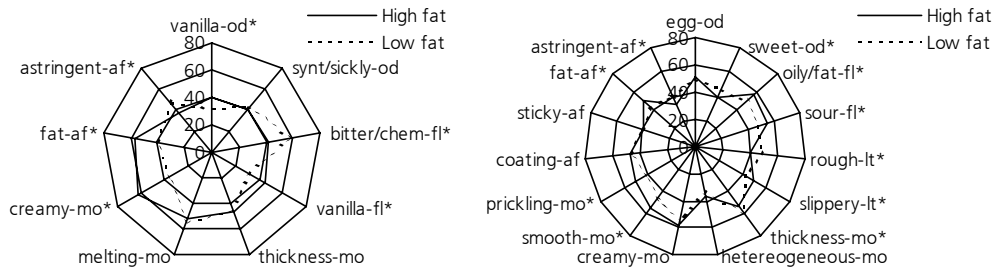


Fig 1. The product differences between high- and low-fat products of custard desserts (a) and mayonnaise (b). *p < 0.05.

Table 3. p-values of the main effects and interactions (ANOVA) for custard desserts and mayonnaise

	Custard				Mayonnaise			
	T prod	T oral	Fat %	Interactions	T prod	T oral	Fat %	Interactions
Vanilla-od	**	-	-	-				
Synth/sickly-od	*	-	-	-				
Egg-od					-	-	-	-
Sweet-od					-	-	*	-
Vanilla-fl	-	-	**	-				
Bitter/chem-fl	**	-	**	-				
Sour-fl					-	-	**	-
Oily/fat-fl					*	-	**	-
Rough-lt					-	-	**	-
Slippery-lt					-	-	**	-
Temperature-mo	***	-	-	-	***	-	***	***
Thick-mo	***	-	-	-	***	-	***	-
Heterogen.-mo					**	*	-	-
Creamy-mo	-	-	***	***	-	-	-	-
Smooth-mo					-	*	*	-
Prickling-mo					-	-	*	-
Melting-mo	***	*	-	-				
Sticky-mo					-	-	-	-
Coating-af					-	-	-	-
Fat-af	*	-	***	**	-	-	**	-
Astringent-af	-	-	*	-	-	-	*	-



Mayonnaise

Table 2 shows the ratings for oral and product temperature and product. Product temperature significantly affected a number of attributes, where a higher product temperature resulted in more oily/fat flavor, temperature and heterogeneous mouth feel, and less thick mouth feel ratings. Oral temperature had significant effects on heterogeneous and smooth mouth feel, where a higher oral temperature resulted in lower ratings. There were large differences in ratings between the high- and low-fat products (Fig 1b). The high-fat mayonnaise was perceived as having less rough lip-tooth feel, and more slippery lip-tooth feel, oily/fat and sour flavor, being thicker, smoother, more prickling and less cold, and having more fatty and less sweet odor and astringent after feel than the low-fat mayonnaise. In addition, the temperature ratings of the low-fat mayonnaise stretched a broader range, with lower ratings than the high-fat mayonnaise. A significant interaction was seen between the product temperature and oral temperature on the temperature ratings, where oral temperature showed opposite effects on cold and hot mayonnaise. At low product temperatures in combination with a hot mouth, the temperature ratings were higher than in combination with a cold mouth.

Viscosity measurements

The viscosities of the custard desserts and mayonnaises were influenced by the temperature. The trend was that the higher the temperature, the lower the viscosity (Fig 2). A decrease in viscosity of about 57 % and 26 % respectively for custard dessert and mayonnaise was seen when raising the product temperature from 10°C to 35°C.

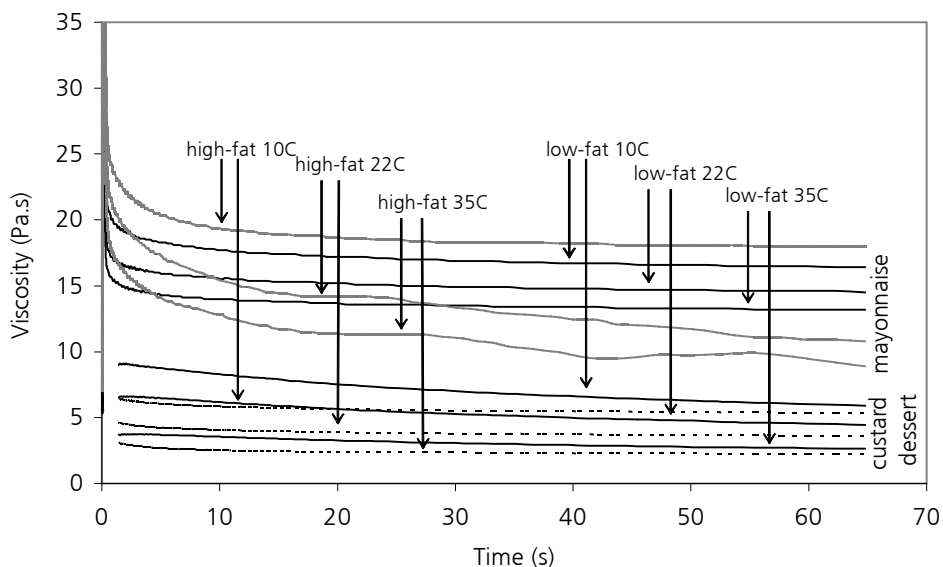


Fig 2. Viscosity measurements of custard desserts and mayonnaises at 10, 22 and 35 °C

DISCUSSION

In this study we investigated the effects of product and oral temperature on odor, flavor and texture attributes in two semi-solids; custard desserts and mayonnaises. Product temperature was shown to have a large effect on the sensory attributes. Oral temperature also affected sensory attributes, but to a lesser extent. This suggests that the physico/chemical characteristics are powerful in eliciting sensations of flavor and texture attributes and that these characteristics are readily altered by a change in temperature.

Mouth rinses of different temperatures were shown to be capable of changing the temperature of the intra-oral mucosa. The maximal temperature difference between mucosa and product was 33°C; this was the case with a heated mouth and a cold product. These findings are in accordance with what others have found when investigating the *in vivo* temperature of teeth during meals (22); (23). These authors reported that the maximal difference in temperature was 29°C and 28°C, respectively.

Odors and flavors

The intensities of odor and flavor attributes were seen to increase with increasing product temperature for custard dessert as well as for mayonnaise. An explanation for this could be that with a higher temperature the odorous compounds became more volatile, resulting in a high concentration of these compounds reaching the receptors in the nose. An additional effect could be that with the temperature induced decrease in thickness, these compounds were more readily released from the matrix. The lower viscosity paired with higher temperature might facilitate the taste compounds to enter the saliva and hence reach the taste buds.

Temperature

The subjects were well able to differentiate between the product temperatures, while only little effect on temperature rating was seen for the oral temperature. There seemed to be an effect of fat percentage, where subjective temperatures of the mayonnaises were rated to be in a narrower range than the custard dessert. A possible explanation could be found in the thermal nature of the products, where due to the low thermal conduction coefficient of oil in comparison to water, a high fat product like mayonnaise insulates better, being perceived as less cold at low temperatures and less warm at high temperatures than custard dessert. This seems plausible since the high-fat mayonnaise exhibited an even more narrow range than the low-fat mayonnaise. These results are in accordance with previous results (24) .

Thickness and melting

The attributes thickness and melting were shown to be strongly affected by an increased product temperature in custard dessert, where thickness decreased and melting increased. The actual viscosity was found to decrease during moderate heating. The viscosity of mayonnaise was less influenced by an increase in temperature and so were the thickness ratings. In accordance to this, perceived thickness has previously been positively correlated to stimulus viscosity (25-27).



Melting was not only affected by the product temperature, but also by oral temperature. This effect seemed to be enhanced after a hot mouth rinse, temporarily raising the temperature of the oral tissue. A possible explanation for this could be the enhanced enzymatic action of salivary α -amylase with increasing temperature. Since custard dessert is a starch based product, the α -amylase has a strong effect on the breakdown of the custard dessert (28) and hence possibly on the sensation of melting. In agreement with this, previous studies (28) have shown that the amount of saliva affected the melting mouthfeel. By adding extra saliva to the product, the melting sensation increased above the level of when water only was added.

Creaminess

There was no significant over-all effect of product temperature on creaminess. However, different patterns could be observed for the high- and low-fat custards, where a high product temperature resulted in less creaminess in high-fat custard and more creaminess in low-fat custard. Creaminess is suggested to be a complex attribute strongly related to thickness and smoothness (29) in addition to a flavor or taste attribute (30;31) This was also observed in modeling analyses, where creaminess of vanilla custard dessert was predicted from a combination of flavors (creamy- and fatty flavor and absence of bitter/chemical and sickly flavors), thickness, fattiness, and (absence of) roughness ratings (32). In the present study viscosity decreased with increasing temperature, whereas fat afterfeel, vanilla odor and flavor increased with a rise in temperature. This increase of certain attributes and decrease of others, all with possible effects on creaminess, might be compensatory, resulting in relatively stable ratings of creaminess irrespective of temperature.

Fat after feel

Fat after feel might be related to the amount of residual food that is left on the oral tissue as coating after the food bolus is swallowed. Considering the increased sensation of fat after feel in low-fat custard as a result of higher product temperatures, it can be hypothesized that at low temperatures part of the fats in custard dessert are present in solid state, but with heating these fats melt and are completely melted at 35° C (33). Melted fat might be more prone than solid fat to spread and to leave a fatty residue behind on the mucosa after swallowing. Another possibility is that a higher temperature weakened the food matrix and made the fat more available to form a fatty coating. This could explain the effect of higher product temperatures on fat after feel ratings of custard dessert. However, since the low-fat custard dessert contained only 0.2 % fat it can be speculated that other ingredients mimicked fat at higher temperatures, e.g. the type of thickener could be of importance. Considering the observed effect of product temperature on fat after feel, one could expect a similar effect of oral temperature. Conversely, a trend was observed where a high oral temperature counteracted this effect, resulting in less fatty after feel. It is suggested that warm mucosa hinders the residual layer of fat from forming, thereby reducing fat after feel. However, the precise mechanism for this effect is unclear.

No effect on fat after feel was seen for mayonnaise. The discrepancy between low-fat custard dessert, and high-fat custard dessert and mayonnaise could be explained by the higher fat content of mayonnaise, which might already saturate fat perception. In addition, the fat present in mayonnaise was already in liquid state and would not change further by a change in temperature.

Product differences

The two product groups showed different effects to changes in temperature. A possible explanation is the largely different composition and physico/chemical characteristics of the groups. Custard desserts are dairy products where the milk is stabilized and thickened by starch and carrageenan, whereas mayonnaise is an oil-in-water emulsion where a network is formed of fat droplets emulsified by egg yolk proteins. The egg yolk proteins also see to that the mayonnaise remains stable. In addition starch, or another thickener, e.g. xanthan, is also often added to commercial mayonnaise to achieve the desired viscosity. As previously discussed, the starch in custard dessert is sensitive to breakdown by amylase, possibly affecting the melting attribute.

There were also large differences in ratings between the high- and low-fat products within each group of products. These differences could be attributed to the varying concentrations of the ingredients, e.g. fat, starch, and other thickeners.

Conclusions

From this study it can be concluded that product and oral temperature influence the perception of certain flavor and texture attributes in semi-solids, with a larger effect on custard desserts than on mayonnaise. For the evaluation of food products, it is therefore important to test the food sensorially at expected serving temperature. It could also be of importance to consider if the food is going to be eaten in combination with e.g. a hot or a cold drink, as this might influence the perception of the product. The fact that variation of product and/or oral temperatures highlights specific flavor/texture sensations may be useful for product development or quality control where one typically wants to focus on certain sensations and ignore others. Further research is needed to fully understand the underlying mechanisms and the effects that physico/chemical properties have on texture perception.



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CHAPTER 9
CHEWING BEHAVIOR AND SALIVA
SECRETION

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ABSTRACT

We determined the salivary flow rate in 16 healthy subjects in rest and while chewing artificial and natural foods (Parafilm, Melba toast with and without margarine, and three different volumes of breakfast cake and cheese). We also determined the duration of a chewing cycle, the number of chewing cycles until swallowing, and the time until swallowing. The physical characteristics of the foods were quantified from force-deformation experiments. The flow rates of the saliva as obtained without stimulation, with Parafilm stimulation, and with chewing on the various foods were significantly correlated. An increase in chewing cycle duration, number of chewing cycles until swallowing, and time until swallowing was observed as a function of the volume of the food. More chewing cycles were required for Melba toast than for an equal volume of cake or cheese. This may be caused by the low water and fat percentage of the Melba toast. The number of chewing cycles and the time until swallowing significantly decreased when the Melba toast was buttered, which may be caused by a facilitation in bolus formation and lubrication of the food. The number of chewing cycles until swallowing was not correlated to the salivary flow rate.

INTRODUCTION

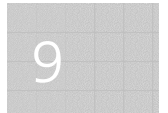
Chewing is the first step in the process of digestion and is meant to prepare the food for swallowing and further processing in the digestive system. During chewing, the food bolus or food particles are reduced in size and saliva is secreted to moisten and lubricate the food. The urge to swallow the food could be triggered by a threshold level in both food particle size and lubrication of the food bolus (1-3).

Subjects with a reduced masticatory performance, due to an inadequate dentition, need more chewing cycles to prepare the food for swallowing than those with a good performance (4-6). Furthermore, they swallow larger food particles (6;7). Thus, subjects with an inadequate dentition compensate for their reduced chewing performance by chewing for a longer period of time and by swallowing larger food particles.

The production of sufficient saliva is indispensable for good chewing. The water in saliva moistens the food particles, whereas the salivary mucins bind masticated food into a coherent and slippery bolus that can be easily swallowed (8). It has been suggested that the swallowing process initiates when the cohesive forces that bind food particles together into a bolus are strongest (3). The important role of saliva for chewing and swallowing is demonstrated by the finding that the number of chewing strokes, hence time in the mouth, needed for swallowing significantly increases after experimentally induced oral dryness (9). Additionally, significantly more saliva is required for oral manipulation of powdered crisp bread than for pieces of crisp bread (10) as the larger surface area of the powder requires more saliva for lubrication and cohesive binding in preparation for deglutition. In a study on rabbits, it was demonstrated that greater amounts of saliva were produced for dry food than for moist food (11). The amount of saliva also plays a role in the chewing of meat, with more saliva being incorporated into a food bolus of tough meat, than into tender meat before the bolus is swallowed (12).

While saliva and chewing have been shown to be interrelated, the relationship between amount of saliva and mastication has not been studied extensively (13). During mastication it is likely that mechanoreceptors in the gingival tissues will be stimulated which may result in salivary flow (14;15). At chewing forces as low as 5% of comfortable chewing forces the masticatory-salivary reflex could already be elicited (14).

The aim of the present study was to investigate the influence of the salivary flow rate on the chewing process. We determined whole saliva flow rates under various conditions: unstimulated and stimulated by chewing artificial and various natural foods. Furthermore, we determined the number of chewing cycles and the time needed to prepare various volumes of food for swallowing. In order to relate amount of saliva not only to volumes of food, but also to physical characteristics of the foods, force-deformation experiments were performed. Widely different types of food were included in the study; dry and crisp Melba toast, sweet and moist cake and fat cheese.



MATERIALS AND METHODS

Subjects

Sixteen healthy subjects (8 males and 8 females) participated in the study. Their age ranged between 16 and 60 years (mean 35 ± 13 years). They all had a natural dentition at least up to the second molars without evident defect of dental structures, periodontal conditions or severe malocclusion. The Ethics Committee of the University Medical Center Utrecht approved the protocol. Written informed consent was obtained from each subject after a full explanation of the experiment.

Test foods

We used the following natural foods: toast (Melba toast, Buitoni, Italy, www.buitoni.com; diameter 5.0 cm, thickness 0.4 cm and volume 7.9 cm^3) with 2 g of margarine spread on one surface (Linera, Unilever, the Netherlands, www.unilever.nl), toast without margarine, three differently sized blocks of breakfast cake (Right, Peijnenburg, the Netherlands, www.right.nl; 9.2, 14.0, and 20.0 cm^3), and of aged Gouda cheese (3.0, 6.0, and 9.0 cm^3). The 3 volumes will be referred to as small, medium and large portions. Table 1 shows the characteristics of these foods. The physical properties of the food samples were tested by crushing the food in a pneumatic bite simulator. This apparatus consists of a probe attached to a pneumatic cylinder. The probe has a conical cusp with a slope of 120 degrees (16). The position of the probe during crushing was monitored by a linear variable differential transformer and the velocity was 1 mm/s. Force-deformation curves were obtained by plotting the data points of the force as a function of the percentage deformation of the food samples. From these curves, the forces and compression percentages were obtained at the yield point. Six samples of each food were measured.

Saliva collection

Saliva samples were collected in 3 different ways: unstimulated, mechanically stimulated, and food stimulated. Firstly, we collected unstimulated saliva to determine a “baseline” flow rate (17). Secondly, stimulated saliva was obtained by chewing on a piece of tasteless Parafilm (0.29 g; Parafilm “M”®, American National Can™, Chicago, IL, USA). Unstimulated and mechanically stimulated saliva were collected over a period of 5 min. Before collection, the mouth was emptied by an initial swallow. At 30-s intervals saliva was expectorated into pre-weighed containers and flow rates (ml/min) were calculated. The weight of saliva in grams was assumed to equal the millilitres of saliva secreted, because the specific density of saliva is close to 1.0 (18). Finally, saliva was obtained by chewing on the various natural foods. Before the experiments, all foods were brought to room temperature (20°C). Margarine was stored at 4°C . We assumed that the saliva produced equals the difference between the weight of the served food and the weight of chewed food that is collected when the subjects are ready to swallow (19). The natural test foods were given to the subjects in a predetermined sequence. The subjects were asked to chew the food in their usual manner until they wanted to swallow. Instead of swallowing they spat out the food bolus into a pre-weighed container. Prior to the

experiments, it was emphasised that all chewed material needed to be recovered. Subjects were instructed to clean their mouths with tongue and cheeks while spitting into the pre-weighed containers and a probe was used to facilitate the removal of trapped particles. Tests were performed twice. In between the stimuli, subjects were allowed to sip water.

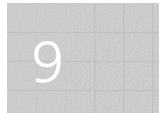
The volume of saliva was determined by subtracting the initial weight of the food from that of the food/saliva mixture. For each food, salivary flow rate was calculated as the volume of saliva secreted, divided by the time the food was in the mouth (ml/min). All samples were collected during the morning, since salivary flow rate shows a circadian rhythm (20;21). In addition, the amount of saliva secreted per gram of food was calculated (ml/g).

Chewing cycles

Masticatory mandibular movements were recorded by an optoelectronic device (Northern Digital Optotrak™; www.ndigital.com) during the chewing of natural test foods, in order to identify individual chewing cycles. The device tracks the 3-dimensional position of two small infrared light emitting diodes (LED's) that were attached to the mandible and the head. By comparing their positions we obtained the movement of the mandible with respect to the head. From the generated plots we determined the number of chewing cycles until the individuals were ready to swallow (swallowing threshold), as well as the average time of each chewing sequence.

Statistical analysis

Repeated-measures analysis of variance (ANOVA; SPSS 9.0; SPSS, Chicago, IL, USA) was applied to test the null hypothesis that there would be no statistical difference among the results obtained for the various food types and volumes. Subsequently, contrasts were determined to study the levels of the within-subjects factors (food type and volume). Furthermore, we tested whether the volume of the food caused a linear increase in saliva flow rate and chewing variables. Pearson correlations were calculated to quantify relationships among the unstimulated and parafilm stimulated saliva flow rates and the flow rates obtained for the various foods. We also tested possible relationships among the salivary flow rate obtained from chewing on a food and the number of chewing cycles needed to prepare that food for swallowing. Equal volumes of the natural foods (melba toast, small portion of cake, and large portion of cheese) were compared for cycle duration, number of chewing cycles and time until swallowing with repeated measures ANOVA.



RESULTS

The means and standard deviations of the force and deformation at the yield point of the various foods are presented in Table 1.

Table 1. Food characteristics

	Density (g cm ⁻³)	Water (%)	Fat (%)	Yield point*	
				Force (N)	Deformation (%)
Melba toast	0.38	5	4.7	16.3 (1.3)	14.0 (4.5)
Breakfast cake	0.59	18	10.2	1.86 (0.24)	27.8 (6.6)
Cheese	1.08	35	31	4.90 (0.88)	20.5 (1.5)

*Means and standard deviations obtained from six measurements

Flow rates and amounts of saliva

Table 2 presents the average values for the flow rates of the saliva as obtained for the various foods. The salivary flow rates for cheese are not presented: these results were unreliable because the cheese could not be fully recovered after chewing as the cheese sticks to the teeth. This factor could even lead to calculated negative values for the salivary flow rate. Repeated-measures ANOVA on the saliva flow rates showed a significant effect for the type of saliva stimulation ($P < 0.001$). Contrast analysis showed that the flow rate obtained without stimulation was significantly lower ($P < 0.001$) than the flow rate obtained from chewing on Parafilm, whereas the flow rate obtained with Parafilm was significantly lower ($P < 0.001$) than the flow rates obtained from chewing food. No significant differences in flow rates were observed among the various foods and volumes. The flow rates of the saliva as obtained without stimulation, with Parafilm stimulation, and with chewing on the various foods were significantly correlated (Table 3). Saliva secreted per gram of food differed significantly among the foods, with toast eliciting the highest levels, followed by toast with margarine, and cake (Table 2). Less saliva per gram was observed for larger volumes of cake.

Table 2. Saliva secretion in response to different foods*

	Unstimulated	Parafilm	Toast	Toast with margarine	Cake (small)	Cake (medium)	Cake (large)
Saliva flow rate (ml min ⁻¹)	0.53 ^a (0.28)	1.40 ^b (0.67)	8.64 ^c (5.06)	7.74 ^c (4.97)	7.97 ^c (5.02)	7.32 ^c (3.97)	7.42 ^c (3.61)
Saliva per gram (ml g ⁻¹)			1.07 ^d (0.53)	0.87 ^c (0.54)	0.40 ^b (0.23)	0.33 ^a (0.21)	0.32 ^a (0.17)

* Means and standard deviations (in parenthesis) obtained from 16 subjects. Values with different superscript letters on a horizontal line are significantly different, where the letter a has the lowest value. ($p < 0.05$).

Table 3. Matrix of Pearson correlations between saliva flow rates obtained with various ways of stimulation for 16 subjects*

	1	2	3	4	5	6	7
1. Unstimulated	-						
2. Parafilm	0.74 ^b	-					
3. Toast	0.57 ^a	0.77 ^c	-				
4. Toast plus margarine	0.72 ^b	0.71 ^b	0.74 ^b	-			
5. Cake (small)	0.50 ^a	0.69 ^b	0.87 ^c	0.81 ^c	-		
6. Cake (medium)	0.71 ^b	0.63 ^b	0.66 ^b	0.92 ^c	0.81 ^c	-	
7. Cake (large)	0.66 ^b	0.66 ^b	0.69 ^b	0.89 ^c	0.89 ^c	0.94 ^c	-

* Superscript letters (two-sided tests): a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$

Chewing characteristics

The average duration of a chewing cycle, the number of chewing cycles and the time until swallowing for the various foods and volumes are presented in Table 4. Repeated-measures ANOVA showed that the duration of a chewing cycle linearly increased as a function of the volume of the food for both cake ($P = 0.003$) and cheese ($P = 0.002$). The cycle duration increased on average by 5 ms (cake) and 12 ms (cheese) for every additional cm^3 of food. Additionally, the number of chewing cycles and the time until swallowing linearly increased with the volume of food that was chewed for both cake ($P < 0.001$) and cheese ($P < 0.001$). On average 1.7 (cake) and 2.1 (cheese) extra chewing cycles were needed for every additional cm^3 of food, whereas the additional chewing time was 1.2 s (cake) and 1.6 s (cheese). Among the 3 foods with equal volumes (melba toast, small portion of cake, and large portion of cheese), we observed significant differences in cycle duration ($P < 0.001$). The cycle duration for cake was smaller than for toast, which was smaller than for cheese. We also observed significant larger values for the number of chewing cycles and time until swallowing for melba toast as compared to cake and cheese. No differences in number and time existed between cake and cheese. The number of chewing cycles and the time until swallowing significantly decreased when the melba toast was buttered ($P < 0.02$).

No significant correlations were observed between the salivary flow rate obtained from chewing on a food and the number of chewing cycles needed to prepare that food for swallowing.

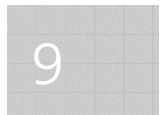


Table 4. Average duration of a chewing cycle, number of chewing cycles, and time until swallowing for the various foods*

	Toast	Toast with margarine	Cake (small)	Cake (medium)	Cake (large)	Cheese (small)	Cheese (medium)	Cheese (large)
Cycle duration (s)	0.64 ^b (0.08)	0.64 ^b (0.09)	0.61 ^a (0.08)	0.62 ^{a,b} (0.07)	0.67 ^b (0.10)	0.64 ^b (0.07)	0.69 ^c (0.10)	0.71 ^c (0.11)
Number of cycles	37.6 ^e (9.9)	32.4 ^d (7.2)	28.4 ^c (7.3)	36.9 ^e (9.8)	46.4 ^f (10.5)	14.4 ^a (3.6)	21.8 ^b (6.3)	27.0 ^c (7.0)
Chewing time (s)	23.8 ^e (5.8)	20.5 ^d (4.5)	17.4 ^c (5.2)	23.0 ^e (6.5)	30.7 ^f (7.2)	9.3 ^a (2.6)	14.7 ^b (3.9)	18.9 ^{c,d} (4.5)

* Means and standard deviations (in parenthesis) obtained from 16 subjects. Values with different superscript letters on a horizontal line are significantly different, where the letter a has the lowest value. ($p < 0.05$).

DISCUSSION

The major functions of the oral phase in response to a meal are the breakage of food into smaller particles by chewing and the addition of saliva, so that a food bolus is produced that can be swallowed. Saliva plays a role in taste sensation, bolus formation and digestion of starch and lipids (8;22;23). We measured whole saliva rather than that of an individual gland, because whole saliva is easy to collect, causes the subjects less discomfort during collection, is readily measurable, and better represents the oral environment (13;24). Whole saliva is a combination of secretions from the submandibular, sublingual, parotid and minor glands. The composition of saliva varies and depends on the type of gland that produces it. Submandibular and sublingual saliva owes its mucous character to the relatively high levels of mucins. These mucins exhibit diverse functions in saliva, among others protection against pathogens (25), dehydration (26), and perhaps more important in this study, lubrication (27). Parotid saliva, on the other hand is practically devoid of mucins and therefore highly serous. It contains high levels of amylase, the enzyme initiating the breakdown of starch in the mouth.

The unstimulated and stimulated saliva flow rates we found (Table 2) were similar to previously reported flow rates (19;28-31). The response to chewing Parafilm was a threefold increase in the salivary flow rate compared with the unstimulated level. The salivary flow rates observed when eating (un)buttered melba toast and 3 volumes of cake ranged between 7.42 and 8.64 ml/min (Table 2). We observed no significant differences among these values. A higher salivary flow rate might have been expected for melba toast as higher bite forces are needed to fragment the toast as it has a higher yield force than the other foods (Table 1) and masticatory force has been reported to influence salivary flow (11;32). However, the higher bite forces are probably only present in the beginning of the chewing process as the toast will be softened by the saliva after a few chewing strokes. Furthermore, a harder product is also chewed for a longer time before deglutition and the salivary flow rate tends to decrease over

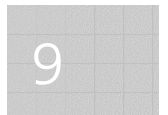
the chewing sequence (33;34). This may counteract the effect of the higher bite forces on salivary flow rate. Due to retention of food in the mouth and inadvertent swallowing, the salivary flow rate will be slightly underestimated (18;19). However, in our study inadvertent swallowing seldom occurred as could be seen from the movement signal of the lower jaw. The flow rates elicited in response to chewing natural foods in our study concur with flow rates reported in previous studies: *e.g.* 3.4 ml/min for chewing gum (18), 6.7 ml/min for rhubarb pie (19), 4.8 ml/min for apple (29), 6.3 – 8.3 ml/min for cookies (33), and 1.8 – 6.9 ml/min for cheese (35). The relatively high salivary flow rates that we observed, may be attributed to the type of food we used. Both melba toast and cake are dry products (Table 1). These products need more saliva in order to moisten the food and form a food bolus that can be swallowed. Indeed, in a study on rabbits, higher salivary flow rates were observed for dry food (dry pellets) than for moist food (pieces of carrot) (11).

The salivary flow rates observed for the natural foods are much higher than for chewing Parafilm. Parafilm is an inert and tasteless material, so it does not cause gustatory secretory stimulation. The effect of gustatory stimulation of foods has been found to be more important than the mechanical stimulation of chewing for the saliva flow rate (19;36). Hence this may explain the much lower flow rate when chewing on parafilm than on natural foods. Furthermore, the Parafilm was chewed for a longer time (5 min) than the natural foods (30 s or less; Table 4). This may lead to lower flow rates as there is evidence of a reduced flow rate with prolonged chewing (33;34).

Significant correlations were observed between the unstimulated flow rate, the stimulated flow rate, and the flow rates elicited by the natural foods (Table 3). Thus, determining the saliva flow rate from either unstimulated chewing or chewing on Parafilm is as good a method for obtaining an indication of the salivary flow as determining the flow rate from natural foods. The Parafilm method may then be preferred, because it is the easiest and cleanest way of obtaining an adequate amount of saliva.

As stated above, we observed no significant differences among the amounts of saliva secreted per minute for the various foods and volumes. In contrast, the amount of saliva secreted per gram of product differed significantly among the different types of foods. Melba toast elicited the highest levels, followed by buttered melba toast and cake. As melba toast contains the lowest percentage of water and fat, this is evidence that a dry product needs more saliva to moisten and form a cohesive bolus suitable for swallowing which is in accordance with previous research (11). As the saliva secretion per minute was not influenced by the various foods, a dry product thus needs a longer time in the mouth to allow for enough secretion of saliva. Our results confirm a previous finding that the chewing time per weight of food is inversely related to the water content of the food (29).

We observed that the average duration of a chewing cycle increased as a function of the volume of the food. Apparently, bolus formation and size reduction of the food during a



chewing cycle take more time for larger food volumes. Furthermore, the number of chewing cycles increased with volume. Equal volumes of cake and cheese were swallowed after the same number of chewing cycles on average. However, an equal volume of melba toast needed more chewing cycles to prepare for swallowing. This may be caused by the very low water and fat percentage of the toast. More saliva may be needed to obtain a food bolus that can be swallowed, and thus more chewing cycles are required. Indeed, the average number of chewing cycles needed before swallowing toast significantly decreased when the toast had 2 g of margarine on it. The margarine facilitates bolus formation and lubricates the food, which makes it easier to swallow.

No significant correlations were observed between the salivary flow rate, while chewing on a food and the number of chewing cycles needed to prepare that food for swallowing. Although large differences in flow rate among subjects are present, as can be seen from the rather large standard deviations (Table 2), these differences do not lead to corresponding differences in the number of chewing strokes. Apparently, subjects are used to their respective amounts of saliva, so that swallowing threshold is not influenced by a subject's amount of saliva. Thus, a subject with a relative large salivary flow does not necessarily swallow the food after a relative small number of chewing cycles. In a recently reported study, individual salivary flow rates did not influence sensory ratings (37). The absence of correlation between flow rate and sensory ratings was explained by the assumption that all the subjects are used to their amounts of saliva and have their own reference, probably a result of experience.

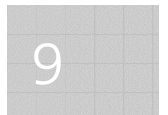
The present data shows an increase in cycle duration and number of chewing cycles until swallowing as a function of the volume of the food. Furthermore, we observed a larger number of chewing cycles until swallowing for foods with less water and fat. A dry product needs a longer time in the mouth to allow for enough secretion of saliva for the formation of a food bolus that can be swallowed, because the salivary flow rate (ml/min) was not larger for a product with less water and fat.

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CHAPTER 10

THE RELATION BETWEEN SALIVA FLOW RATE AFTER
DIFFERENT STIMULATIONS AND THE PERCEPTION OF
FLAVOR AND TEXTURE ATTRIBUTES IN CUSTARD
DESSERTS

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Physiology and Behavior 78: 165-169, 2003



ABSTRACT

Salivary flow rates were measured at rest and after three types of stimulation; odor, parafilm chewing and citric acid. The highest flow rate was elicited by citric acid followed by parafilm and odor, while the lowest flow rate was unstimulated. In order to investigate if and how the amount of saliva a subject produces influences the sensory ratings, the four types of salivary flow rates were correlated with sensory ratings of three different types of vanilla custard dessert. No significant correlation could be found between any of the salivary flow rates and the sensory ratings. A subject with a larger volume of saliva in the mouth during eating did not rate the foods differently from a subject with less saliva present. The same pattern was seen for all types of stimulation. This finding could indicate that subjects are used to their respective amounts of saliva to such a degree that the differences in sensory ratings between subjects cannot be explained by the inter-individual difference in saliva flow rate.

INTRODUCTION

Many studies have focused on the effects of food and other stimuli on the flow rate and composition of saliva. Different stimuli are known to affect salivary secretion. The stimulation can be either intra-oral, such as taste or mechanical stimulation, or extra-oral, such as ambient factors, odors or expectations (1). Salivary flow is often stimulated by non-food mechanical stimulation (2;3). Chemical stimulation, by a solution of citric acid elicits a strong response (4) and other oral irritants also induce the flow of saliva, probably to protect the mucosa (5). Other authors have focused on the stimulation by taste solutions (6-8) or real food stuffs (9-13). A combination of gustatory and mechanical stimulation is seen to elicit high saliva flow rates (10;13). Depending on the oral stimuli the type of food can alter saliva characteristics, such as flow and composition. Soft foods exert only little mechanical stimulation in the mouth, but can act as odor or chemical stimuli. The odor of vanilla custard desserts is highly distinctive for the product.

Saliva is expected to be involved in our perception of the taste, flavor and texture of foods. The effects of saliva on the food leading to changes in perception are plentiful. The mixing of saliva with food can have a diluting effect, moreover it can influence the flavor release (14). The action of the enzyme alpha-amylase present in the saliva, which initiates the digestion of starch, could also result in a drop in the perceived thickness of the food. Furthermore saliva acts as a buffering system (15-17), affecting the degree to which we perceive sourness (18). In addition to that, the large salivary proteins can influence the lubrication (19) and hence perhaps the perception of attributes such as smoothness and astringency (20). However, subjects presented with identical stimuli often differ in their reports on the strength of sensations. The question thus arises: Could the amount of saliva present in the mouth at the time of rating account in part for the variations in sensations? The relation of parotid saliva flow and the perception of sensory attributes specific for different stimuli has been studied (21). They found positive correlations between the parotid flow rate and cohesiveness of crackers and adhesiveness of peanut butter.

The purpose of this study was to determine the salivary flow rates elicited under different stimulations; unstimulated, mechanical, chemical and odor, and to examine the relationship between flow rates of saliva stimulated in different ways and the perception of selected flavor, texture and after feel attributes of semi-solids.

MATERIALS AND METHODS

Subjects

Twenty-two subjects, 14 females and 8 males, aged between 19 and 33 years (average 24.2 years) participated in the study. The subjects had previously been screened for a well functioning olfactory and taste ability and had received extensive training in the use of sensory

odor, flavor, texture and after feel attributes for custard desserts. The subjects gave informed consent and were compensated financially for their participation and divided into a morning and an evening group based on their availability.

Saliva

Whole saliva flow was measured during rest, after stimulation by odor (AH vanilla vla, AH, the Netherlands), during mechanical stimulation (chewing Parafilm®, American National Can, Greenwich, CT, US), and during chemical stimulation (Citric acid monohydrate, Merck, Darmstadt, Germany). Saliva was collected on four separate occasions, where only one type of salivary stimulus was presented per session. Each single subject was always tested on the same time of the day. During five-minute periods saliva was spat at 30-second intervals into pre-weighted containers and flow rates (ml/min) were calculated. Parafilm was chewed during the whole collection period. The custard odor was administered by holding a bowl of custard under the subjects' nose during five minutes' stimulation. Three droplets of 4% citric acid were applied to the tongue at 30-second intervals.

Food stimuli and sensory testing

Three different commercially available vanilla custard desserts, one based on soybeans and the other two based on low and high fat milk, were used in this study. The custards were purchased in a local supermarket on the same day of each session to assure freshness. The custard dessert used in this study – called “vla” in Dutch- is a popular product in the Netherlands. In addition to milk or soybeans they contain sugar, modified starch, hydrocolloids like carrageenans, and colorants and aromas. The custard desserts consist of two phases, a continuous aqueous one and a dispersed one made of starch granules and fat globules that are stabilized by protein containing membranes.

Eight sensory attributes, including flavors (vanilla and bitter/chemical), mouth feel (temperature, thickness, melting and creaminess), and after feel, the oral sensation remaining after swallowing, (here fat and astringent) were rated for all three custards. These attributes were selected as a representative sub-set of the 35 attributes developed previously for vanilla custard desserts by a Quantitative Descriptive Analysis (QDA) panel.

The subjects were seated in sensory booths with appropriate ventilation and lighting. During one 2-hour session subjects were presented with triplicates of each of the three stimuli. First, the custard was first smelled and odor attributes were rated. Next, ingested custard was rated on the taste/flavor and mouth feel attributes. Finally, the custard was swallowed and the two after feel attributes rated. Acquisition of the subjects' responses was done by computer on a 100-point response scale using FIZZ software (Biosystemes, 1998). Panel testing took place at the sensory facilities of TNO-Nutrition and Food Research in Zeist, the Netherlands.

Data processing and analysis

The sensory data was collected and analyzed by FIZZ software (Biosystemes, 1998). Repeated measures ANOVAs were performed with SPSS (SPSS inc., Chicago, IL) on the salivary data with type of stimulation and product, gender and time of day as factors. The same software was used to correlate the salivary flow rate for different types of stimulation within subjects and to correlate sensory data with the salivary flow rates. $p < 0.05$ was considered significant.

RESULTS

Saliva collection

The mean salivary flow rates after the various stimulations are depicted in Table 1. It appears that there was a significant difference between the saliva flow rates after the various stimulations, with the citric acid eliciting the highest flow rate, followed by the parafilm chewing and the custard odor stimulation, while the unstimulated saliva flow rates were the lowest.

The differences between male and female subjects for the unstimulated, mechanical and chemical stimulation conditions were found not to be significant. In addition, no significant difference could be seen between the morning and the evening group. The flow rates of unstimulated, odor and parafilm stimulated saliva were significantly and positively correlated, while there was no significant correlation between salivary flow rates elicited by citric acid stimulation and any of the other salivary flow rates (Table 2).

Table 1. The average salivary flow rates at rest and after stimulation with vanilla custard dessert odor, Parafilm chewing, and citric acid.

	Flow rate ml / min (S.D.)			
	Unstimulated	Odor	Mechanical	Chemical
Female	0.35 (0.17)	0.52 (0.20)	1.06 (0.40)	2.06 (0.68)
Male	0.45 (0.18)	0.50 (0.24)	1.22 (0.52)	2.69 (1.92)
Morning	0.37 (0.17)	0.47 (0.22)	1.04 (0.24)	2.49 (1.48)
Evening	0.41 (0.19)	0.53 (0.20)	1.16 (0.54)	2.0 (0.77)
Total	0.38 (0.18) ^a	0.51 (0.21) ^b	1.12 (0.45) ^c	1.87 (1.21) ^d

Different letters indicate significant differences, $p < 0.05$.

Table 2. The correlation between sensory ratings and salivary flow rates (FR)

	1	2	3	4	5	6	7	8	9	10	11
1. FR unstimulated	-										
2. FR odor stimulated	0.76**	-									
3. FR Parafilm	0.52*	0.51*	-								
4. FR citric acid (4 %)	0.30	0.25	0.46	-							
5. Bitter/chemical –fl	0.08	0.25	0.23	0.01	-						
6. Vanilla-fl	0.02	0.23	0.12	-0.15	0.10	-					
7. Temperature-mo	0.06	-0.19	-0.06	-0.02	-0.12	0.03	-				
8. Thickness-mo	0.08	0.03	0.17	-0.07	0.01	0.39	0.29	-			
9. Melting-mo	0.02	0.13	0.30	-0.14	0.26	-0.02	-0.33	-0.43	-		
10. Creamy/soft-mo	-0.14	-0.03	-0.07	-0.21	0.20	0.55*	-0.03	0.26	0.04	-	
11. Fat-af	-0.13	-0.01	-0.05	-0.39	0.47*	0.44	-0.01	0.30	0.26	0.69**	-
12. Astringent-af	0.45	0.29	0.19	0.31	0.10	-0.22	0.09	-0.12	0.21	-0.58**	-0.18

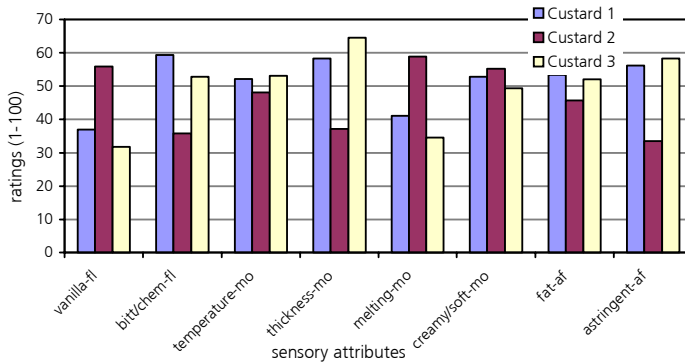
The three attribute groups are flavor (fl), mouthfeel (mo), and afterfeel (af).

*p < 0.05

**p < 0.01

Sensory data

The mean sensory ratings for each attribute are found in Fig 2. Results indicate considerable differences between the types of custard for most attributes. The high fat dairy custard was rated as having more vanilla flavor, being less thick, more melting, creamier and less astringent than its low fat and the soy-based counterparts. The low fat dairy custard was rated as thicker



and more astringent than the high fat and the soy-based custards. The soy-based custard was rated as having a stronger bitter/chemical flavor than its dairy based counterparts. Temperature was rated the same for all three custards.

Fig 1. The average sensory ratings of eight flavor (fl), mouthfeel (mo), and afterfeel (af) attributes for three vanilla custard desserts. Custard 1 is soy-based, custard 2 is based on high-fat, and custard 3 is based on low-fat milk.

Relationship salivary flow rate and sensory ratings

No significant correlations were found between any of the salivary flow rates and the ratings of the sensory attributes (Table 2). When comparing the sensory ratings of high and low salivary flow rate subjects, again no significant difference was found for any of the sensory attributes and ways of saliva stimulation between the two groups.

DISCUSSION

In this study we have investigated the salivary flow rates elicited by different types of stimulation and their possible relations with flavor, texture and after feel sensations.

The results of the salivary flow rates show large differences in the potency of the different stimulations on the flow rate, with citric acid stimulation eliciting the highest flow rates and unstimulated saliva the lowest. Our results are in agreement with previous studies, where the amount and composition of whole saliva have been seen to vary markedly with the type and intensity of the stimulus (unstimulated: 0.4 ml/min (13), 0.3 ml/min (19), and 0.4 ml/min unpublished observations; parafilm stimulated: 0.9 ml/min (19), and 1.3 ml/min unpublished observations, and citric acid: 1.7 ml/min (19)). Often saliva was collected directly from one type of gland, e.g. parotid or submandibular, therefore it is difficult to compare these results with ours. The amount and composition of saliva can also vary with the time of day (22-24). Variations in individual flow rates can be as high as 50% over a day due to circadian rhythms (25;26), and can increase up to fourfold from resting levels upon stimulation (19;27;28). This is taken into consideration in this study, where all the saliva collections and sensory ratings took place on the same time of the day for each subject. However, in our study there was no difference in flow rates between the morning and the evening group. Since all the saliva from one subject was collected at the same time of day, those results are based on a morning vs. evening comparison on group level, and not on the differences within individuals. The present study showed no significant gender effect on salivary flow rates, in accordance with other studies (4;12;13), unpublished observations). The rather low amounts of saliva elicited by custard odor indicate a weak, but real anticipatory effect, which has also been shown by other investigators (1). They showed that the sight of foods containing sour or pungent ingredients (lemon and pizza) elicited a higher flow rate than more neutral, but highly palatable foods. Vanilla custard is a soft and neutral food eliciting only little mechanical and chemical stimulation, and its odor does not give any indications to the contrary. The anticipatory reaction is therefore that little saliva will be needed to protect the mucosa or break the food down, resulting in rather low flow rates.

When correlating salivary flow rates with sensory ratings, we saw no significant relationship between the two parameters. This is in agreement with the absence of difference in sensory ratings seen between the groups of subjects with high and low salivary flow rates. Subjects with a high saliva flow rate did not rate the foods differently, with stronger or less strong sensations, from a subject with less saliva present, irrespectively of the type of stimulation. This is in

agreement with the fact that subjects with high flow rates for one type of stimulation, mostly also had high flow rates for the other types of stimulation, except for the case of citric acid stimulation. The subjects are apparently used to their amounts of saliva. The absence of correlation between flow rate and sensory ratings may be explained by the assumption that all the subjects have their own reference and that the ratings are relative rather than absolute, and probably a result of experience. In this explanation it is supposed that each subject uses a certain product as a reference (e.g. for maximum creaminess) and the attributes of other products are always referred and compared to this standard. In doing this all the ratings can be seen as relative ratings and a subject with low saliva flow-rates might keep the same distance between the products as a subject with high saliva flow-rates. To be able to investigate the absolute effect of amount of saliva on texture perception, saliva levels within one subject should be altered. Studies are planned in which saliva will be added to the product prior to ingestion. The fact that others have found a correlation between salivary flow rate and sensory texture attributes (21), while we failed in doing so, can be attributed to the difference in products and experimental set-up. In this study all the seventeen sensory attributes were rated for only one type of product, while the subjects in the study of Guinard et al. (21) rated a wider range of products, including solids and only one product specific attribute per product.

We conclude that the explanation for inter-personal differences in sensory ratings of semi-solids could not be found in the different salivary flows within the normal range of these subjects. From these results, however, we cannot say what the effect would be during extreme salivary conditions, as in pathological circumstances.

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CHAPTER 11

A COMPARISON OF ADDED SALIVA, α -AMYLASE
AND WATER ON TEXTURE PERCEPTION IN
SEMI-SOLIDS

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ABSTRACT

The effect of adding saliva or a saliva-related fluid (α -amylase solution and water) to custard prior to ingestion on the sensory ratings of odour, flavour, lip-tooth-feel, mouth-feel and after-feel sensations was investigated. Saliva had previously been collected from the subjects and each subject received his/her own saliva. Sixteen subjects from a trained panel assessed 17 flavour and texture attributes of soy- and milk-based custard desserts. Immediately prior to administration, two different volumes (0.25 and 0.5 ml) of three different saliva-related fluids (saliva, α -amylase solution and water) were added to the product. The added volumes represented an approximately 33 % and 66 % increase of the volume of saliva present in the mouth during ingestion. The results show that addition of a fluid affected the mouth-feel attributes of melting, thickness and creamy. Melting was the only attribute on which the type of fluid had an effect, where saliva elicited a stronger melting effect than the α -amylase solution and water. The volume of the added fluid affected a number of attributes (thick and creamy mouth-feel and fatty after-feel). It can be concluded that in general the sensory attributes of semi-solids were relatively stable. Mouth and after-feel sensations were partly affected, while odour, flavour, and lip-tooth-feel sensations were not affected by an increase in volume of saliva or other saliva-related fluid during ingestion.

INTRODUCTION

Saliva is expected to be involved in our perception of taste, flavour and texture of foods. A number of researchers have investigated the effect of saliva on selected attributes (1-6). The effects of saliva on food leading to changes in perception may be plentiful. The mixing of saliva with food can influence flavour release (7-11); moreover, taste and flavour substances can become diluted (7;12). The action of the enzyme α -amylase present in saliva, initiating the digestion of starch, could also result in a drop in perceived thickness of the food. Furthermore saliva acts as a buffering system (13-15), affecting the degree to which we perceive sourness (16). In addition to that, the large salivary proteins may influence the lubrication (17) and hence possibly the perception of attributes such as smoothness and astringency (5;18).

Previous work in this laboratory has shown that there was no relation between a subject's unstimulated and stimulated salivary flow rates, and his/her sensory ratings of semi-solids (1). Furthermore, a subject with a high flow rate for one type of stimulation, in most cases also had a high flow rate for the other types of stimulation. A possible explanation for the absence of correlation between salivary flow rate and sensory ratings could be that subjects have their own references and that ratings are relative rather than absolute, and probably a result of experience. This explanation is based on the assumption that each subject uses a certain product as reference (e.g. for maximum airiness) and the attributes of other products are referred and compared with this standard. In doing this, all ratings can be seen as relative and subjects might keep equal differences among products irrespective of the subjects being high or low salivators. Subjects are apparently used to the volumes of saliva in their mouths, and the systems thus seem to be calibrated for the subjects' individual salivary levels during eating. Assuming this is the case, it can be hypothesized that an artificial increase in the amount of saliva mixing with food could influence the perception of the food. The reason for this is that any addition of saliva would disturb the equilibrium of the system slightly. To test this hypothesis, fluid was added to food prior to administration to the subjects. By adding fluids to the mouth and stimuli during eating, the amount of fluid present in the mouth was increased to levels higher than the endogenous levels. To test the effect of different components of saliva, three different fluids were chosen; whole saliva previously collected from the subjects, a solution of α -amylase at physiological concentration to study the effect of starch breakdown, and pure tap water to investigate the dilution effect only.

The purpose of this study was to investigate what effect a disturbance of the in-mouth equilibrium by an artificial increase of saliva, or one of its liquid components, has on the perception of sensory attributes in semi-solids during ingestion.

METHOD & MATERIALS

Subjects

Sixteen subjects, 10 females and 6 males, aged between 20 and 36 years (average 26.1 years) participated in the study. The subjects had previously been screened for olfactory and taste

disorders and had received extensive training in the use of sensory odour, flavour, mouth-feel and after-feel attributes for custard desserts. The subjects gave informed consent and were compensated financially for their participation, and divided into a morning and an evening group based on their availability. Each single subject was, however, always tested on the same time of the day.

Added fluids

The three fluids used in this study were saliva, α -amylase solution and pure tap water. Saliva was collected prior to the present experiment by letting the subjects chew on a 5x5 cm square sheet of tasteless paraffin (Parafilm® American National Can, Greenwich, CT, US) during five-minute periods and spit every 30 s into pre-marked containers. The collected saliva was centrifuged at 11000 xg for five minutes and the supernatant was stored at -20°C. Prior to the experiment saliva was thawed and kept on ice. Saliva from different subjects was held strictly separate throughout the study, and during the assessment the subjects received only their own saliva. A 50 U/ml solution of human α -amylase (product number 10092, Fluka BioChemika, Buchs, Switzerland) was prepared freshly prior to each experiment and kept on ice during the experiment. The chosen α -amylase activity was of the same magnitude as mean values found by Mackie and Pangborn (19) (60-70U/ml in whole saliva) and Froelich et al. (20) (50-60U /ml in parotid saliva). It has been reported that the salivary contribution to a tastant is approximately 0.8 ml (21). This amount was also shown to be the residual volume after swallowing (22). We chose to increase this amount of saliva present in the mouth with approximately 33 and 66 percent in this study. With a bite size of vanilla custard dessert of 6 ml, this amounts to 0.25 ml and 0.5 ml of fluid added to the spoon and product.

The fluids were put onto spoons with disposable pipettes, in the volumes of 0.25 and 0.5 ml and were left for a few minutes to allow for adjustment to room temperature.

Food stimuli and sensory testing

Foods

Two different commercially available Dutch vanilla custard desserts, one based on soybeans and the other on high-fat milk, were used in this study. The custard desserts were purchased in a local supermarket on the same day of each session to assure freshness. The custard desserts were stored and administered at 10°C, which is the normal serving temperature in the Netherlands.

Attributes

Eighteen sensory attributes, including odours (almond and synthetic/sickly), flavours (vanilla and bitter/chemical), tooth-lip feel (astringent and smooth), mouth-feel (temperature, thickness, airy, melting, prickling, smooth, heterogeneous and creaminess), and after-feel (coating, sticky, fat and astringent), were rated for both custards. Tooth-lip feel is the sensation that arises when rubbing the upper lip against the upper teeth, and after-feel is the sensation remaining after swallowing. The definitions of the rated attributes are given in Table 1. These attributes were selected as a representative sub-set from a set of 35 attributes developed

previously for vanilla custard desserts by a Quantitative Descriptive Analysis (QDA) panel (23).

Table 1. list of 18 descriptive terms related to odour-, flavour and lip-tooth-, mouth- and after-feel for vanilla custard dessert.

Attribute	Definition
Odor (od)	
Vanilla	Intensity of the odour of vanilla.
Synthetic/sickly	Artificial, sickly odour of custard.
Flavor/taste (fl)	
Bitter/chemical	Degree in which the taste of a product is bitter.
Vanilla	Intensity of vanilla flavour.
Lip - tooth-feel (lt)	
Rough	The rough sensation elicited when rubbing the tongue against the front teeth and inside of the lip.
Slippery	The slippery sensation elicited when rubbing the tongue against the front teeth and inside of the lip.
Mouth-feel (mo)	
Temperature (colder-warmer)	Foods may elicit different temperature sensations while presented at the same physical temperature. Sensation is sensed during first contact between food and tongue
Thickness	Represents the thickness of the food in the mouth after the food is compressed via up- and down motions of tongue against palate.
Airy	Food is perceived by the tongue as airy/foamy and disintegrates easily after the food is compressed by the palate.
Melting (slow - quick)	Food becomes thin in the mouth and spreads throughout the mouth at different rates.
Prickling	A prickling, tingling feeling sensed by the tongue, typically associated with carbonated drinks.
Smooth	Degree to which the food contains granules detected by moving the tongue along the palate. Jelly is an example of a smooth product.
Heterogeneity	A product is simultaneously perceived as thick and thin (or "cloudy") in the mouth while the food is manipulated.
Creamy	Range of sensation typically associated with fat content such as full and sweet taste, compact, smooth, not rough, not dry, with a velvety (not oily) coating. Food disintegrates at a moderate rate.
After-feel (af)	
Coating	The residual layer of food after swallowing or expectoration that produces a velvety sensation.
Sticky	Foods leave a sticky feeling in the whole mouth, which is difficult to remove.
Fat	Food leaves a fatty/oily feeling in the mouth after swallowing.
Astringent	Food leaves an astringent taste and feeling in the mouth, typically caused by products like wine, nuts and spinach.

Anchors: If not indicated: very little – very much. The order of the attributes per category is based on the temporal order at which the attributes are perceived during manipulation.

Procedure

The subjects were seated in sensory booths with appropriate ventilation and lighting. During 2-hour sessions on two separate days, subjects were presented with triplicates of all the stimuli. Either 0.25 ml or 0.5 ml of one of the fluids, or nothing as a control, was put on a spoon and on top of that 6 ml of one of the custards, whereafter the spoon was immediately administered to the subject (Fig 1). All combinations of fluids, volumes and custards were administered in random order. The custard was first smelled, after which the odour attributes were rated. Next, ingested custard was rated on flavour and mouth-feel attributes. Subjects kept the stimuli in the mouth during 4-5 seconds, which previously had been observed to be the time they

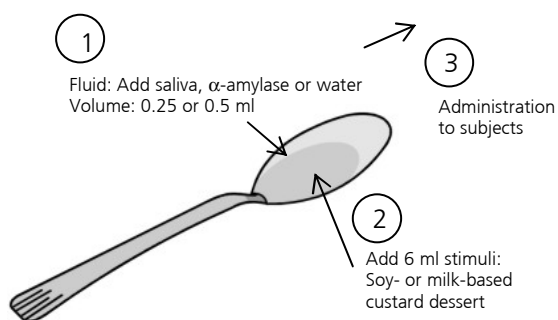


Fig 1. Schematic of the experimental setup.

normally kept the stimuli in the mouth while assessing the same group of attributes (unpublished data). Finally, the custard was swallowed and two after-feel attributes were rated. Acquisition of the subjects' responses was done by computer on a 100-point VAS response scale. Panel testing took place at the sensory facilities of TNO-Nutrition and Food Research in Zeist, the Netherlands.

Data processing and analysis

The sensory data were collected and analyzed by FIZZ software (1998, Biosystèmes, Couternon, France). Repeated-measures ANOVAs were performed with Greenhouse-Geisser as correction factor on data averaged across triplicates (SPSS 9.0 SP 4M, SPSS inc., Chicago, IL). Treatment (control, saliva, α -amylase solution and water), volume (0.25 and 0.5 ml) and type of product (soy-based and milk-based custard desserts) were included as within-subject factors. $p < 0.05$ was considered significant.

Physical measurements

Breakdown measurements were performed with a rheometer (Physica MCR300, Paar Physica, Stuttgart, Germany), during 60s at 150 rpm, as mixing at this rotational speed was found to resemble mixing in the mouth reasonably closely. Eleven grams of custard dessert at 20°C was placed in a serrated cup with a modified vane. Prior to starting the measurement, 0.5 ml of water, α -amylase solution (50 U/ml) or saliva was added to study the effect of mixing and saliva induced breakdown.

RESULTS

Mean ratings of the sensory attributes for the custard desserts, treatments and volumes are shown in Table 2. When investigating the effect of adding a fluid to the food on the ratings of sensory attributes, we saw the following: There was a significant over-all effect of treatment on the mouth-feel attributes melting ($F(3, 45) = 7.9, p = 0.001$), thickness ($F(3, 45) = 6.5, p = 0.000$) and creamy ($F(3, 45) = 15.04, p = 0.004$) (Fig 2). With the addition of a fluid the custards were perceived as being more melting, less thick and less creamy in comparison to the control. A comparison of the effects of the three fluids reveals that the addition of saliva increased the rated melting more than the water and α -amylase solution did ($F(2, 30) = 4.7, p = 0.027$), but no such effect could be seen for the ratings of thickness and creamy, where all three liquids elicited the same effect. The volume of added fluid had significant effects on the ratings of creamy ($F(1, 15) = 5.4, p = 0.034$) and thick mouth-feel ($F(1, 15) = 8.36, p = 0.011$), and fatty after-feel ($F(1, 15) = 7.77, p = 0.014$). In all three cases the addition of a large volume (0.5 ml) resulted in a more pronounced decrease in sensations than the addition of a small volume (0.25 ml). An interaction was observed between treatment and product for heterogeneous mouth-feel ($F(2, 30) = 4.1, p = 0.029$). With α -amylase solution, the soy-based custard was perceived as more heterogeneous than the milk-based custard, whereas this relationship was reversed for the other treatments.

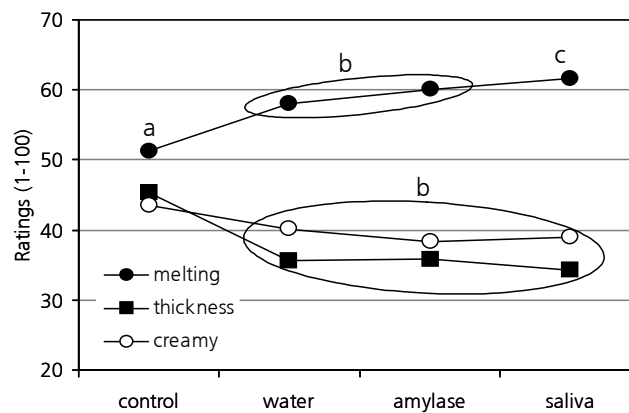


Fig 2. The effects of treatment on melting, thick and creamy mouth-feel. Points designated different letters are significantly different.

Besides these attributes (melting, thick, creamy and heterogeneous mouth-feel and fatty after-feel), the addition of fluids, be it saliva, α -amylase solution or water, had no significant effect on the ratings of the odour, flavour and after-feel attributes of custard desserts (Table 2).

There was a difference in the ratings of the two products where the soy-based custard was rated as having more bitter/chemical flavour ($F(1, 15) = 10.6, p = 0.005$), less vanilla flavour ($F(1, 15) = 12.0, p = 0.003$), and being thicker ($F(1, 15) = 11.6, p = 0.004$), less melting ($F(1, 15) = 29.7, p = 0.000$) and less smooth ($F(1, 15) = 10.2, p = 0.006$) than the dairy custard.

The physical measurements (Fig 3.) show that by only mixing the custard desserts during 60 s, some structural breakdown took place. Structural breakdown was slightly enhanced when water was added. An addition of α -amylase solution induced fast and efficient structural breakdown, and this effect was even stronger when the custard dessert was mixed with saliva. The same relationship was seen for both custards desserts tested. After addition of fluid the torque initially decreased followed by an immediate increase, which in the figure can be seen as humps, lasting until about 7 s. This indicates that the mixing of fluid into the bulk was toilsome, which might be the result of slip. The soy-based custard dessert had a higher resistance to stirring and mixing than its milk-based counterpart.

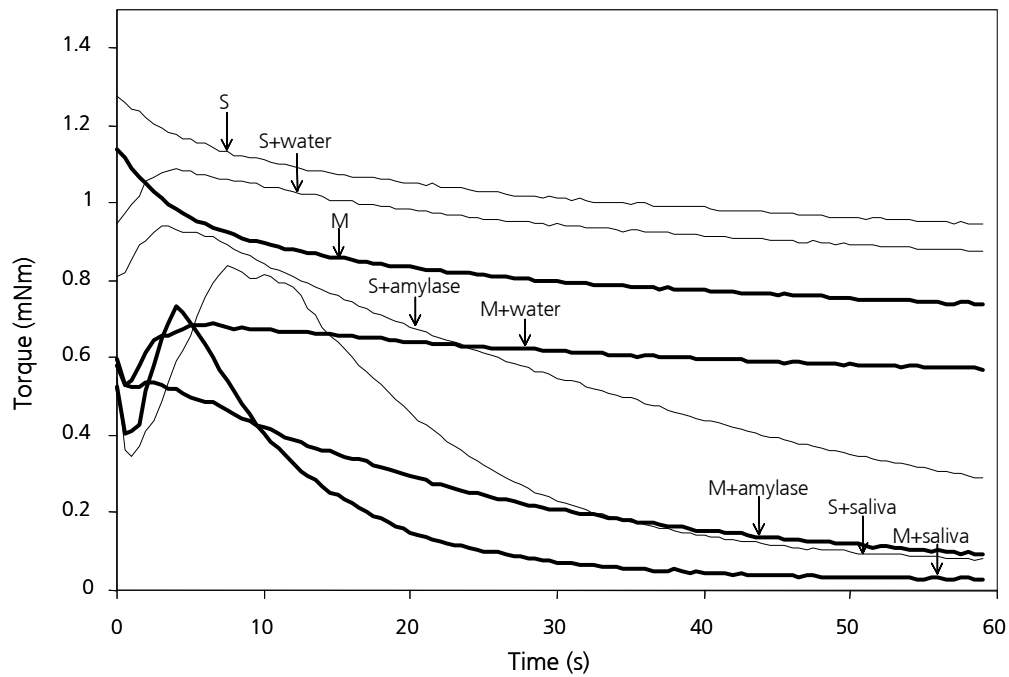


Fig 3. *In vitro* breakdown measurements of soy-based (S, thin lines) and milk-based (M, thick lines) custard desserts mixed with water, α -amylase solution and saliva.

Table 2. Mean ratings and S.D. of the sensory attributes for soy-based (S) and milk-based (M) dessert at four treatments and three volumes.

Product	Treatment	Volume (ml)	almond-od*	synt/sickly-od	bitter/chem-fl *	vanilla-fl *	rough-lt	slippery-lt	temp-mo	thick-mo */#/†	airy-mo	melting-mo */#	prickling-mo	smooth-mo *	hetero-mo€	creamy-mo #/†	coating-af	sticky-af	fatty-af †	astringent-af
S	Control	0	33.5 (19.3)	32.4 (19.5)	52.7 (20.6)	32.7 (13.1)	34.5 (20.8)	44.7 (20.1)	41.3 (12.1)	48.7 (18.6)	20.8 (11.8)	47.0 (17.7)	18.1 (10.8)	60.3 (24.9)	21.8 (16.0)	40.9 (22.2)	35.2 (16.7)	29.1 (16.9)	30.3 (18.0)	38.0 (18.5)
		0.25	34.3 (17.8)	33.8 (23.4)	49.5 (17.4)	34.0 (12.1)	31.0 (17.6)	45.0 (20.4)	44.3 (15.0)	42.9 (15.6)	19.3 (10.8)	51.2 (18.3)	16.5 (8.4)	59.8 (25.7)	26.9 (17.3)	39.0 (20.0)	35.8 (18.5)	30.7 (18.4)	30.8 (17.7)	41.3 (16.6)
			34.5 (17.5)	35.1 (19.2)	48.3 (20.9)	33.2 (13.1)	31.8 (17.9)	47.5 (18.4)	42.1 (13.4)	38.8 (16.2)	21.0 (12.5)	56.9 (17.3)	16.3 (9.8)	61.4 (26.0)	26.0 (17.5)	37.7 (22.4)	34.3 (16.7)	26.2 (14.3)	27.3 (17.0)	39.4 (19.5)
		0.25	35.2 (17.4)	38.1 (18.5)	54.6 (20.1)	36.0 (18.3)	32.9 (17.2)	45.1 (22.3)	43.2 (11.8)	42.3 (16.4)	22.3 (12.2)	52.9 (18.7)	17.2 (13.2)	63.0 (24.3)	27.1 (17.0)	39.3 (19.8)	33.3 (18.7)	27.2 (16.8)	29.7 (17.3)	40.7 (19.1)
			31.8 (19.4)	39.6 (23.6)	52.5 (19.2)	37.2 (18.0)	29.7 (19.4)	46.7 (22.9)	44.0 (12.2)	39.4 (13.9)	22.2 (12.2)	55.8 (17.3)	16.9 (9.3)	65.3 (24.7)	28.0 (17.7)	34.2 (17.8)	32.5 (14.9)	25.0 (13.8)	31.1 (15.6)	42.0 (16.7)
		Amylase	0.5	31.8 (19.4)	39.6 (23.6)	52.5 (19.2)	37.2 (18.0)	29.7 (19.4)	46.7 (22.9)	44.0 (12.2)	39.4 (13.9)	22.2 (12.2)	55.8 (17.3)	16.9 (9.3)	65.3 (24.7)	28.0 (17.7)	34.2 (17.8)	32.5 (14.9)	25.0 (13.8)	31.1 (15.6)
	35.5 (19.8)			32.9 (20.2)	53.5 (20.8)	32.8 (16.3)	28.3 (15.4)	48.5 (18.5)	41.1 (13.3)	40.1 (17.2)	21.3 (12.8)	58.3 (17.2)	17.2 (13.1)	63.3 (21.9)	24.9 (16.4)	39.5 (20.9)	33.7 (16.4)	22.9 (11.5)	32.2 (17.2)	41.0 (18.4)
	0.25		32.0 (19.8)	34.2 (20.2)	52.0 (20.8)	33.7 (16.3)	33.2 (15.4)	46.3 (18.5)	45.7 (13.3)	37.1 (17.2)	23.0 (12.8)	57.5 (17.2)	15.9 (9.3)	64.2 (21.9)	25.3 (16.4)	35.5 (20.9)	31.8 (16.4)	25.1 (11.5)	28.3 (17.2)	42.1 (18.4)
			32.0 (21.3)	34.2 (19.3)	52.0 (17.2)	33.7 (20.4)	33.2 (19.8)	46.3 (20.2)	45.7 (12.5)	37.1 (16.4)	23.0 (16.9)	57.5 (16.7)	15.9 (9.3)	64.2 (24.1)	25.3 (16.0)	35.5 (21.7)	31.8 (15.5)	25.1 (13.2)	28.3 (15.5)	42.1 (18.5)
	0.5		25.5 (14.5)	29.5 (18.2)	35.8 (20.3)	45.6 (20.7)	26.8 (13.8)	44.6 (22.6)	41.2 (11.0)	42.0 (17.5)	26.9 (18.0)	55.5 (18.3)	14.7 (7.8)	64.3 (23.0)	23.8 (14.0)	46.1 (23.0)	31.9 (16.8)	25.9 (11.0)	30.0 (16.5)	37.4 (20.8)
			25.7 (16.6)	27.5 (18.0)	36.2 (22.4)	46.7 (17.3)	23.4 (15.0)	49.3 (22.1)	41.0 (9.9)	31.9 (15.2)	27.7 (21.3)	61.5 (14.9)	15.1 (7.1)	66.7 (21.7)	28.4 (18.5)	42.7 (20.5)	32.2 (16.6)	24.2 (12.0)	27.2 (14.2)	33.3 (16.8)
	M	Control	0	25.5 (14.5)	29.5 (18.2)	35.8 (20.3)	45.6 (20.7)	26.8 (13.8)	44.6 (22.6)	41.2 (11.0)	42.0 (17.5)	26.9 (18.0)	55.5 (18.3)	14.7 (7.8)	64.3 (23.0)	23.8 (14.0)	46.1 (23.0)	31.9 (16.8)	25.9 (11.0)	30.0 (16.5)
0.25			25.7 (16.6)	27.5 (18.0)	36.2 (22.4)	46.7 (17.3)	23.4 (15.0)	49.3 (22.1)	41.0 (9.9)	31.9 (15.2)	27.7 (21.3)	61.5 (14.9)	15.1 (7.1)	66.7 (21.7)	28.4 (18.5)	42.7 (20.5)	32.2 (16.6)	24.2 (12.0)	27.2 (14.2)	33.3 (16.8)
			24.0 (15.6)	27.3 (21.3)	35.2 (21.9)	42.4 (18.4)	21.3 (11.3)	49.7 (25.9)	39.3 (15.0)	28.8 (13.5)	28.5 (20.7)	62.2 (17.7)	14.6 (6.5)	62.5 (27.0)	28.4 (19.1)	41.4 (20.6)	33.6 (19.5)	24.5 (11.9)	27.0 (14.5)	32.8 (16.7)
0.25			23.0 (16.4)	28.5 (16.5)	39.1 (24.6)	48.2 (16.4)	25.2 (13.9)	48.2 (21.9)	41.9 (12.4)	33.6 (15.3)	27.6 (21.0)	63.4 (15.3)	14.9 (8.1)	65.5 (24.7)	25.8 (15.7)	40.8 (19.6)	30.8 (17.2)	29.2 (15.0)	29.2 (12.8)	36.5 (19.3)
			24.2 (15.5)	27.0 (19.8)	35.4 (20.7)	46.5 (21.4)	25.9 (15.1)	53.4 (20.0)	40.0 (14.2)	28.4 (14.5)	30.1 (20.2)	67.8 (12.7)	15.4 (7.8)	66.6 (23.3)	26.4 (17.8)	39.0 (20.6)	29.2 (15.0)	20.7 (10.1)	27.3 (13.4)	36.4 (16.7)
Amylase			0.5	25.4 (15.0)	28.5 (16.6)	39.0 (22.6)	45.6 (17.6)	27.3 (15.8)	51.6 (21.7)	41.3 (11.9)	33.2 (15.5)	26.1 (19.5)	67.5 (12.6)	15.8 (8.9)	66.5 (26.0)	25.3 (16.5)	43.1 (21.6)	33.3 (15.5)	24.2 (15.5)	30.4 (17.8)
		23.5 (16.2)		29.7 (20.1)	34.7 (18.1)	47.0 (24.0)	22.2 (12.7)	52.4 (21.8)	42.7 (12.2)	26.6 (14.9)	27.6 (22.4)	63.4 (20.7)	15.1 (8.2)	69.8 (19.9)	26.3 (21.8)	37.9 (21.8)	32.8 (18.7)	23.7 (15.5)	28.0 (17.6)	32.5 (18.8)

Values in parentheses are S.D. * Significant effect of product; # Significant effect of treatment; † Significant effect of volume; € Significant interactions.

DISCUSSION

In this study the effects of added saliva or saliva-related fluid on sensory attributes in custard dessert were studied.

The odour of vanilla custard is highly distinctive for the product and such soft products need not be chewed. Consequently, the saliva most resembling the type excreted during eating of custard is probably the saliva obtained after stimulation with vanilla custard odour (1). Saliva excreted then is not only stimulated by the odour itself, but also by the anticipation, since the subjects already knew they would receive custard dessert and were used to this product. In spite of this, we chose to use mechanically stimulated saliva in this study for two reasons: 1. The slimy character of saliva caused by mucins, is suggested to influence the perception of mouth-feel attributes. Results from this laboratory (unpublished data) have shown that the mucin concentrations of odour and mechanically stimulated saliva were not significantly different. 2. Large volumes of saliva were required for this study. Due to the relatively low flow rate of odour stimulated saliva, collection of the amounts needed would have caused the subjects excessive discomfort.

The fluid was allowed to adjust to room temperature during a few minutes. However, adding a comparatively large volume of cold custard dessert on top, which then was entered into the warm mouth, probably overruled the initial temperature of the fluid. In addition, previous results (24;25) have shown that the temperature of oral mucosa is of very little importance in comparison to product temperature for ratings of sensory attributes of custard desserts.

The food was added to a spoon covered with fluid. The reasons for covering the spoon with fluid first and then adding the product, instead of mixing the fluid with the product prior to administration were two-fold. Firstly, this mimicked the situation *in vivo*, where the oral mucosa is covered with a thin layer of saliva when the food is ingested, whereafter it is mixed. Secondly, if the saliva and α -amylase solutions were to be mixed with the custard prior to ingestion, the starch would rapidly be broken down by the α -amylase, causing a fast decrease in viscosity.

The results of this study reveal that the addition of saliva, or one of its aqueous components, to custard desserts has some effect on the ratings of sensory odour, flavour, tooth-lip feel, mouth-feel and after-feel attributes. Thickness and melting were two of the attributes on which a 33 or 66 percent increase of saliva or saliva-related fluid during ingestion had the largest effect. One possible explanation for this could be found in the nature of the products. Custard desserts are semi-solids thickened by starch. Thickness is defined by the subjects as the initial thickness, thus rated as one of the first attributes. With the addition of fluids the perceived thickness was decreased. This is in accordance with the *in vitro* breakdown measurements in Fig 3. The fact that the perceived effect was equally strong for water as for α -amylase solution and saliva, and that a larger fluid volume increased the effect is evidence that the decreased sensation was mainly due to dilution.

Melting is defined as the rate of decrease in thickness and spreading of the product in the mouth (Table 1). The fact that for all the added liquids, the rating of melting increased above baseline in this study suggests that it was a dilution effect. Starch is broken down by the salivary enzyme α -amylase, which might explain why the attribute melting was affected more by saliva than by water. The question then remains, why saliva affected melting more than an α -amylase solution. One possibility is that the activity of the enzyme was too low in the solution. Even though the *in vitro* measurements showed that α -amylase in the concentration we used is very potent and instantaneously starts the breakdown of starch in custard dessert, the effect is slower than for saliva. A possible reason for this is that the α -amylase in water solution is less active than in saliva. Supporting this, the presence of chloride ions was shown to be essential for α -amylase to reach full activity (26). Studies performed with mice, indicated that the activity of α -amylase in saliva was higher than the activity of α -amylase in the gland. It can therefore be speculated that other components of saliva, for example hydrolysing enzymes, or products originating from micro-organisms, can also influence the activity of α -amylase (personal communication Prof. Dr. van Nieuw Amerongen).

The creamy sensation decreased when a larger volume of fluid, irrespective of the type, was added to the product. This is in accordance with previous results showing that creamy is related to thickness (27-29) (unpublished data), where thickness up to a certain level increased creaminess. Due to the increased dilution effect of the product by a larger volume of fluid, the thickness, and hence the creaminess, were decreased in this study.

A larger volume of fluid also decreased the fatty sensation remaining after swallowing. An explanation for this could be that the larger volume of aqueous liquid on the outer layer of the product shielded the mucosa against the formation of a fatty coating and enhanced oral clearance of the fat.

Interestingly, the sensations of flavours were not altered by the addition of a fluid. It has been shown (7) that water decreased, while α -amylase increased, the flavour release due to breakdown of the matrix. Perhaps the dilution effect and the effect of mechanical or chemical breakdown were counteracting. Alternatively, the concentrations of flavour in these products were relatively high, resulting in strong sensations, which were not altered by the relatively slight dilution by the addition of fluids.

The two custard desserts were sensorially different, as can be seen from the ratings. There were significant differences in ratings between the products for the attributes: bitter/chemical and vanilla flavour, thick, melting and smooth mouth-feel, and astringent after-feel. While there were large differences in ratings between the products, many of the attributes were not affected by the addition of fluid. This was the case even when the amount of fluid in the mouth at time of ingestion was doubled. The volumes added may have been too small to influence the sensation significantly.

Even though custard desserts are products thickened by starch, the effects of added saliva and α -amylase solution on sensory perception seem to be limited, with the exception of melting

mouth-feel. Whether this holds for other types of products we do not know. It can be speculated that the amount of α -amylase naturally present in saliva of healthy subjects is such that adding extra enzyme does not affect sensory perception. Alternatively, since thickness is one of the first attributes to be rated, the thickness ratings may be established before the effects of breakdown become prominent. Another explanation is that the residence time in the mouth (for custard dessert around 4 s) was too short to allow for a significant breakdown of starch in the bulk by α -amylase, hence a small decrease in viscosity. During this limited time, the interaction between the product and the saliva, or α -amylase solution is suggested to take place mainly on the surface of the product, affecting the attribute melting, but not thickness. This suggests that the attribute melting is related to surface properties of the product. As shown in Fig. 3, breakdown measurements *in vitro* of these two types of custard dessert mixed with water or saliva show a large difference in the breakdown between water and saliva. From this we can conclude that saliva is very potent in breaking down the structure of the product, thus decreasing the viscosity. Mixing and measurement artefacts during the first seconds make it difficult to study the exact effects as a function of time. Although the time scale and way of mixing is not the same as in the mouth, the results from the *in vitro* study suggest that during the confined time of custard dessert in the mouth (4-5 s), the effects of breakdown were undoubtedly present, but limited. The breakdown by α -amylase in the mouth would then be more important for breakdown of food with longer residence time in the mouth, such as bread and other cereals. During mastication of solids the mixing could also be more vigorous, thereby enabling the enzyme to come in contact with more starch particles, not confined to the surface.

Since neither the saliva flow rate, nor an added volume of saliva seem to elicit substantial differences in sensory ratings in the product stimuli, it can be concluded that the odour, flavour and texture attributes of custard desserts are only partially influenced by the amount of saliva during ingestion.

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CHAPTER 12

THE EFFECT OF SALIVA COMPOSITION ON
TEXTURE PERCEPTION IN SEMI-SOLIDS

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ABSTRACT

Saliva is expected to be of significance for the perception of food stimuli in the mouth. Mixing the food with saliva, including breakdown and dilution, is considered to be of large importance for semi-solids as these products are masticated without chewing. It is known that there are large variations in composition of saliva originating from different glands and different subjects. In this study we investigated how variations in salivary characteristics affect sensory perception. Eighteen trained subjects participated in the study. Saliva was collected at rest and during three types of stimulation (odor, parafilm chewing and citric acid), and flow rates were determined. The collected saliva was analyzed for protein concentration, buffer capacity, mucin level and α -amylase activity. The salivary components measured in this study varied considerably among subjects, but also within subjects as a result of different means of stimulation. Variations in salivary components were correlated with sensory perception of a number of flavour, mouth feel and after feel attributes in the semi-solids mayonnaise and custard dessert. Total protein concentration and α -amylase activity were observed to correlate most strongly with texture perception.

INTRODUCTION

Saliva is expected to be of importance for the perception of food stimuli in the mouth. It can play a role by initial breakdown of food (1;2), by affecting flavour release (3-7), dilution of flavours and tastes (3) (8), precipitation of proteins by tannins e.g. resulting in a sensation of astringency (9) (10), lubrication of the oral tissue (11;12), facilitating manipulation of food in the oral cavity and swallowing, and by transport of taste compounds to the taste buds. These examples indicate the value of saliva for the appreciation and acceptance of food. Saliva consists for more than 99 % of water and contains a large number of organic and inorganic constituents (13) (14). Saliva is therefore considered important and it seems plausible that both the amount and composition of saliva present in the mouth while eating are of importance. A previous study has shown results contradicting the importance of amount of saliva (15). Saliva flow rate of healthy subjects failed to show any correlation with sensory sensations of vanilla custard dessert. A possible explanation for this is that the subjects are used to their own amounts of saliva and their ratings are compared with an internal standard. The continuation of that study was to add extra saliva to increase the normal level that the subjects were used to (1). In that study different components of saliva were compared: in addition to saliva, water and an α -amylase solution were added to food immediately prior to ingestion. The results indicate that many effects of saliva on flavour and texture sensations are attributed to dilution, since the three fluids produced similar results. For attributes concerned with thickness and melting of the product, however, saliva and α -amylase were more potent. Addition of saliva produced the strongest sensation for melting, indicating that saliva exhibits additional effects on the product. Obviously there are components in saliva, other than water, that affect the food while in the mouth. Of the numerous possible compounds, we made a selection of three to analyze, all hypothesized to play a role either in oral breakdown or perception of food: α -amylase, proteins and mucins. In addition, buffer capacity of the saliva was measured, as it is thought to be of importance for taste perception and pH dependent reactions. The mucin analyzed in this study is the MUC5B, also known as MG1. MUC5B is a very large mucosal glycoprotein present in a mucous layer that covers and protects the oral cavity (11;16-18). The mucins exhibit diverse functions in saliva, among others protection against pathogens (11;19;20) and dehydration (11;21;22), and perhaps more important in this study, lubrication (12;23-25).

α -Amylase initiates starch digestion in the mouth. By cutting the long carbohydrate strands at the alpha (1-4) binding between glucose residues, the starch is reduced in its ability to bind water and the result is a lower viscosity of the product. Sensorially, α -amylase is shown to influence the sensation of melting in semi-solids (1) (de Wijk et al. unpublished data).

Proteins play a possible role in taste chemoreception and in the perception of astringency, viscosity, and other mouth feel attributes (9;26;27).

Saliva acts as a buffering system (28) (29) (30), affecting the degree to which we perceive sourness (31). The buffering effect of saliva is attributed largely to bicarbonate/carbonate ions,

and to a lesser extent to phosphate-ions and proteins present in saliva (32), neutralizing acids ingested or produced by micro-organisms in the mouth.

Semi-solids are a group of products masticated without chewing. Therefore mixing with saliva, including structure breakdown and dilution is considered to be of relatively large importance in mastication of these products. Consequently, the components of saliva are suggested to play a considerable role in mastication and perception. It is known that there are large variations in composition of saliva originating from different glands, and different subjects (33), but it is not known how these variations in salivary characteristics affect sensory ratings.

The aim of this study was to investigate: firstly, the composition of saliva after different stimulations; and secondly, the influence of salivary composition on flavour and texture sensations in custard and mayonnaise.

METHOD AND MATERIAL

Subjects

Eighteen healthy adults (6 male and 12 female, with an average age of 24.5 years) participated in the study. The subjects were selected on the basis of a well-functioning olfaction and taste perception, and had received extensive training in the use of sensory odour, flavour, texture, and after feel attributes for semi-solids. Each person gave informed consent and was paid for their participation.

Saliva

Collection

Whole saliva flow was measured during rest and after three types of stimuli: 1. after stimulation by odour (AH vanilla vla, AH, the Netherlands), 2. during mechanical stimulation (chewing Parafilm®, American National Can, Greenwich, CT, US), and 3. during chemical stimulation (citric acid monohydrate, Merck, Darmstadt, Germany), as described elsewhere (15). Saliva was collected on four separate occasions, where only one type of stimulus was presented per occasion. To avoid circadian variations, each subject was always tested on the same time of the day. All four types of saliva were collected in the following way: During five-minute periods saliva was spat at 30-second intervals into pre-weighted containers and flow rates (ml/min) were calculated. The three stimuli were applied as follows: 1. custard odour was administered by holding a bowl of custard under the subjects' nose during five minutes. 2. Parafilm was chewed during the whole collection period. 3. Three droplets of 4% citric acid were applied to the tongue at 30-second intervals. After collection saliva was centrifuged at 10,000 rpm for five minutes to remove buccal cells and oral micro-organisms. The clear supernatant was stored at -20°C until further use.

Analysis of saliva

Total salivary protein was measured by the bicinchoninic acid protein assay (34) with bovine serum albumin as standard. This assay is described in detail by Bosch *et al.* (35).

Mucin concentration was determined by ELISA as described by Veerman *et al.* (36). The mucin levels were compared to standard saliva, a mixture of saliva from a large number of subjects and stimulations, e.g., a result of 200 means that the mucin level in that sample is 200% of the standard saliva mixture.

Buffer capacity was measured by a modified version of Ericsson's method (30). 200 μ l saliva was mixed with 600 μ l HCl (0.0033 M). pH measurements (PHM 240 Labmeter, Radiometer, Copenhagen, DK) started immediately after mixing and read when stable or after 60 seconds, whichever occurred first.

α -Amylase activity was assayed by EnzChek Amylase kit (E-11954, Molecular Probes, Leiden, the Netherlands, www.probes.com) according to the protocol. The kit contains a starch derivative that is labelled with a dye to such a degree that fluorescence is quenched. α -Amylase catalysed hydrolysis relieves this quenching, yielding brightly fluorescent dye-labelled fragments. The accompanying increase in fluorescence is proportional to α -amylase activity and was monitored with a fluorescence microplate reader (Fluostar, Galaxy, BMG laboratories, Offenburg, Germany). A number of changes to the protocol were made as described below: Human salivary α -amylase (art. Nr. 10092, Fluka, Buchs, Germany) was used as control enzyme in the following concentrations: 0.5-1.0-1.5-2.0-2.5-3.0-3.5-4.0 U/ml to produce a calibration curve. 0.1 % BSA was added to the provided 1x buffer. The saliva samples were used in a dilution of approximately 1:100,000. The reaction was measured every minute during 18 minutes and the enzyme activity per minute was read on the calibration curve.

Food stimuli and sensory testing

Two types of commercially available semi-solids were used in this study. A vanilla custard dessert (starch- and hydrocolloid-thickened dairy product, 0.2 % fat) and a mayonnaise (oil-in-water emulsion, 36 % fat) were chosen for their different fat contents and sensory profiles. A trained descriptive sensory panel affiliated with this research group had established these profiles (37). The attributes used in the studies were selected as a representative sub-set from a set of attributes developed previously for vanilla custard desserts and mayonnaises by a Quantitative Descriptive Analysis (QDA) panel (37;38)(39). The selected attributes were divided into four subsets, based on their functional character. These subsets were flavors, tooth-lip feel, mouth feel, and after feel (Table 1). Tooth-lip feel is the sensation arising from rubbing the tongue against the upper lip and upper front teeth and after feel is the oral sensation remaining after swallowing. Some attributes were overlapping between the custard dessert and the mayonnaise.

Table 1. List of descriptive terms related to flavor, lip-tooth feel, mouth feel, and after feel for custard dessert and mayonnaise.

Attribute	Product group	Definition
Flavour/taste (fl)		
Bitter/chemical	Custard	Degree in which the taste of a product is bitter.
Vanilla	Custard	Intensity of vanilla flavor.
Oily/fatty	Mayo	Degree in which the taste of a product is oily/fatty
Sour	Mayo	Intensity of the sour flavor (as in vinegar, lemon, or dairy products).
Lip-tooth-feel (lt)		
Rough	Custard	The rough sensation elicited when rubbing the tongue against the front teeth and inside of the lip.
Slippery	Custard	The slippery sensation elicited when rubbing the tongue against the front teeth and inside of the lip.
Mouth-feel (mo)		
Temperature (colder-warmer)	Custard & Mayo	Foods may elicit different temperature sensations while presented at the same physical temperature. Sensation is sensed during first contact between food and tongue
Thickness	Custard & Mayo	Represents the thickness of the food in the mouth after the food is compressed via up- and down motions of tongue against palate. For less viscous products like dressings, information is also obtained from the rate of spreading of the product throughout the mouth.
Airy	Custard	Food is perceived by the tongue as airy/foamy and disintegrates easily after the food is compressed by the palate.
Melting (slow - quick)	Custard	Food becomes thin in the mouth and spreads throughout the mouth at different rates.
Smooth	Custard & Mayo	Sensation: property of moving food bolus. Examples of smooth products are jelly. An example of a grainy product is semolina. Mechanism: sensation is perceived by moving the food with the tongue along the palate or teeth.
Heterogeneity	Custard & Mayo	A product is simultaneously perceived as thick and thin (or "cloudy") in the mouth while the food is manipulated
Creamy	Custard & Mayo	Range of sensation typically associated with fat content such as full and sweet taste, compact, smooth, not rough, not dry, with a velvety (not oily) coating. Food disintegrates at moderate rate.
Prickling	Mayo	A prickling, stinging, biting sensation that one wants to extinct, typically perceived at the top and side surfaces of the tongue.
After-feel (af)		
Creamy	Custard & Mayo	A velvety (not oily) coating remaining after swallowing
Sticky	Custard & Mayo	Foods leave a sticky feeling in the whole mouth, which is difficult to remove.
Fat	Custard & Mayo	Food leaves a fatty/oily feeling in mouth after swallowing.
Astringent	Custard & Mayo	Food leaves an astringent taste and feeling in the mouth, typically caused by products like wine, nuts and spinach.

Anchors, where not indicated: very little – very much. The order of attributes within categories and product groups is based on the temporal order in which they are perceived during mastication, as indicated by the QDA panel.

The subjects were seated in sensory booths with appropriate ventilation and lighting. During a 1.5-hour session subjects were presented with triplicates of each of the two stimuli. Ingested stimuli were rated on the flavour, lip-tooth feel and mouth feel attributes. Finally, the stimuli were swallowed and the after feel attributes rated. Acquisition of the subjects' responses was done by computer on a 100-point response scale using FIZZ software (Biosystèmes 1998, Couternon, France). Panel testing took place at the sensory facilities of TNO Nutrition and Food Research in Zeist, the Netherlands.

Data processing and analysis

Repeated-measures ANOVAs were performed (SPSS 9.0, SPSS inc., Chicago, IL) on the salivary data with type of stimulation, composition and gender as factors. The same software was used to correlate the salivary composition for different types of stimulation with sensory data. $P < 0.05$ was considered significant.

RESULTS

Saliva

In figs 1 and 2 the results of the saliva flow rates and composition are given. Different types of stimulation produced significantly different salivary flow rates: citric acid elicited the largest amounts of saliva, followed by mechanical stimulation (parafilm), odour stimulation, and unstimulated saliva. The composition of saliva as a response to the four types of stimulation varied significantly. Protein concentration was highest in unstimulated saliva, followed by saliva stimulated by odour, chewing, or citric acid. The highest buffer capacity was found in the mechanically stimulated saliva, followed by resting and odour stimulated saliva. Mucin concentration was higher in resting than in any type of stimulated saliva. For α -amylase activity there were no significant variations among saliva obtained by different types of stimulation, nevertheless the activity in mechanically stimulated saliva was close to significantly ($p = 0.06$) higher than in odour stimulated saliva. The only significant gender effect was found in total protein concentration in odour stimulated saliva, where males had a higher concentration than females.

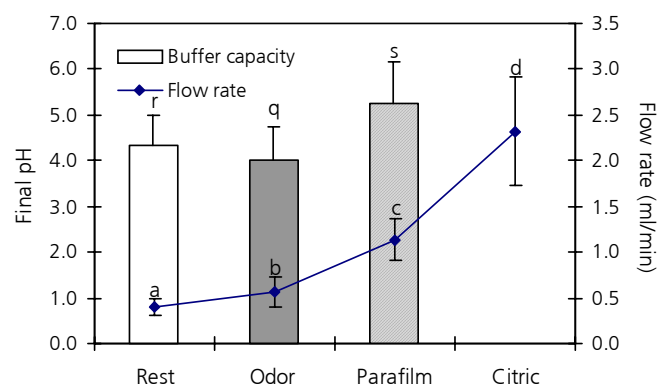


Fig 1. Means and SDs of salivary flow rates, and final pH for resting saliva and saliva stimulated by vanilla odor, Parafilm chewing and citric acid. Points designated different letters are significantly different. $P < 0.05$

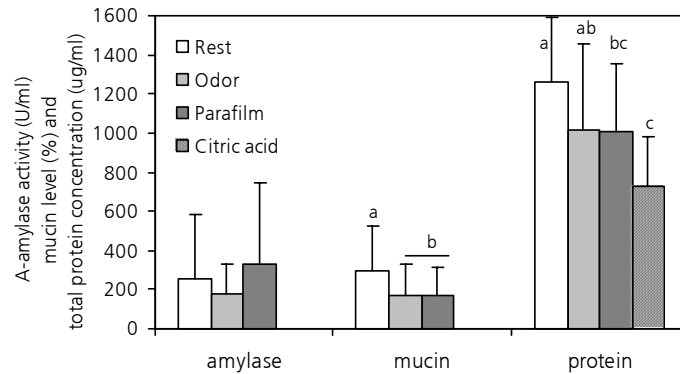


Fig 2. Means and S.D. of α -amylase activity, mucin level and total protein concentration in saliva collected at rest and during three types of stimulation. Bar designated different letters are significantly different. $P < 0.05$.

Saliva and sensory

Correlations of salivary parameters and sensory ratings for custard dessert and mayonnaise are given in table 2.

Custard dessert

The sensory attributes of custard dessert correlated mainly with total protein concentration and α -amylase activity. The composition of mechanically stimulated saliva and odour stimulated saliva seem to be strongly correlated to perception.

Subjects with a high total protein concentration reported low ratings for bitter flavours, slippery lip-tooth feel and fatty after feel.

Mucin levels were negatively correlated with creamy mouth feel.

α -Amylase activity exhibited a negative correlations with vanilla flavour, slippery lip-tooth feel, thick mouth feel, and creamy after feel.

Mayonnaise

One general observation is that the sensory attributes of mayonnaise seem to correlate mainly with total protein concentration of saliva. In respect to the type of saliva, unstimulated saliva and odour stimulated saliva showed the most correlations with perception.

Protein concentration was negatively correlated with oil and sour flavours, thick, smooth, and sticky and fatty after feels. This means that a subject with a high total salivary protein concentration had relatively lower ratings of those attributes.

Buffer capacity was positively correlated with heterogeneous mouth feel.

The mucin level was positively correlated with heterogeneity and negatively correlated with prickling mouth feel.

α -Amylase activity was negatively correlated with prickling mouth feel and creamy after feel, hence a subject with high enzymatic activity had a reduced sensation of those attributes.

Table 2. Significant correlations of salivary parameters and sensory ratings for custard dessert and mayonnaise. $P < 0.05$ was considered significant. Only significant correlations were included, of which at least two correlations of the other salivary stimulations were similar, though not always significant.

			bitter-fl	vanilla-fl	astringent-lt	slippery-lt	temperature-mo	thick-mo	airy-mo	melting-mo	prickling-mo	smooth-mo	heterogeneity-mo	creamy-mo	creamy-af	sticky-af	fatty-af	rough-af				
Custard dessert	Total protein conc.	Rest																				
		Odor																				
		Para CA	-0.7			-0.7														-0.7		
	Buffer capacity	Rest																				
		Odor																				
		para CA																				
	Mucin level	Rest																				
		Odor																				
		Para																			-0.6	
	A- amylase activity	Rest																				
		Odor																				
		Para																				
Mayo nnaise	Total protein conc.	Rest																				
		Odor																				
		Para CA		-0.6	-0.7																	
	Buffer capacity	Rest																				
		Odor																				
		para CA																				
	Mucin level	Rest																				
		Odor																				
		Para																				
	A- amylase activity	Rest																				
		Odor																				
		Para																				

DISCUSSION

Different means of stimulation were potent in eliciting varying amounts of saliva flow rate. This is in accordance with previous results (15;40-43).

Surprisingly, no significant differences were seen in α -amylase activity among the types of stimulation. During mechanical stimulation much of the saliva originates from the parotid gland. Parotid saliva exhibits high α -amylase activity (44). This was also observed in the present study, where mechanically stimulated saliva exhibited high amylase activity, although not completely reaching significance ($p = 0.06$). The secretion of saliva from the parotid gland is strongly increased in response to food (45). As the main function of α -amylase is to initiate breakdown of starch, the logical place to be stored and produced is in the glands that react primarily to food and chewing. Saliva collected during citric acid stimulation showed that the flow rate was increased dramatically.

Mucin levels were higher in unstimulated saliva than in odour- or mechanically stimulated saliva. This is in accordance with (24), in which is stated that at rest whole saliva consists predominantly of saliva secreted from the submandibular gland. Between meals the main function of saliva is to protect the oral mucosa from desiccation, infection and wear during breathing and talking (46). To achieve this, saliva needs to be viscous and have good lubricating capacities, which is accomplished by mucins (11).

Buffer capacity was highest for the mechanically stimulated saliva. This is in agreement with (24) (47) who showed that the buffer capacity of parotid saliva is directly correlated with the flow rate and with the bicarbonate concentration as primary buffering substance in stimulated saliva. Following this reasoning, saliva stimulated by citric acid should have had the highest buffer capacity. Under our conditions, however, whole saliva was contaminated with the applied citric acid and the saliva had already used parts of its buffer capacity to handle the acid applied during stimulation. Total protein concentration was highest for resting saliva and decreased with increasing degree of stimulation, indicating that with increasing flow rate a protein dilution effect occurs.

Salivary components

A number of salivary components were correlated with sensory attributes of the mayonnaise and custard. The saliva that is mixed with food during mastication is a combination of different types of glandular secretions. Prior to eating and perhaps during the first bites, the saliva is of the resting type. When subjected to the odour of vanilla custard and during sampling the saliva is presumably more like the odour stimulated saliva. The basic taste qualities present in custard (sweet and bitter) and mayonnaise (sweet, sour and salt) are known to be associated with a reflex parotid salivary secretion (48). In addition, although the products are soft, some masticatory movements may elicit parotid saliva to be secreted, adding saliva of the mechanically stimulated type. Since mayonnaise is a rather sour product, some saliva of the

type secreted as a response to citric acid stimulation might be present during sensory assessment. Hence the exact combination and composition of saliva during eating of the products in the present study is unknown. We therefore found it relevant to look only at correlations which, however not all significant, were similar for at least three of the salivary stimulations.

Protein concentration

High concentrations of protein were correlated with low flavour ratings in both products. One possible conjecture is that proteins bind the flavour compounds and in that manner decrease flavour release. Furthermore a high protein concentration could possibly induce a decrease in viscosity of the product and lead to a low thickness sensation. The mechanisms for this are unknown, but one could speculate on enzymatic breakdown in addition to the action of α -amylase, or proteins exerting some kind of competitive action with e.g. fat.

Slippery lip-tooth feel was stronger in subjects with saliva having low protein concentration. A speculation is that with a low protein concentration, the viscosity of the custard decreases less (see above) and a more viscous product might stick better to the hard oral surfaces and result in a slippery sensation.

Fatty after feel is less strongly sensed by subjects with a high protein concentration. The proteins might weaken the matrix of the mayonnaise hindering the formation of a fatty layer on the mucosa. Another possibility is that the proteins might compete with fat, or fatty-coating producing compounds for space on the oral mucosa (personal communication M. Paques), hence reducing sensation of fatty after-feel. Thus, the environment or state of mucosa is suggested to be of importance. This was also observed in a previous study (49), where high oral temperatures resulted in lower fatty after feel ratings than low oral temperatures.

Buffer capacity

Buffer capacity correlated with heterogeneity, but the mechanism is not understood. Heterogeneity is defined as being a sensation of inconsistent mouth feel when advancing the tongue through the product. The feeling can also be described as “cloudy” or “flocky”. Perhaps a change, a slight increase in the pH of the rather sour product, due to buffering action of saliva, locally changes the properties of the mayonnaise and hence increases the heterogeneous character of the product.

Mucin level

The mucous, viscous and stringy character of mucins probably impair the mixing of saliva with product in the mouth, resulting in a strong sensation of heterogeneity.

α -Amylase activity

High α -amylase activity was correlated with a reduced sensation of vanilla flavour. This is in accordance with previous results from de Wijk et al. (unpublished), where α -amylase in various concentrations was added to the product prior to ingestion. They raised the possible explanation that due to instant enzymatic breakdown the custard was less viscous, resulting in

less surface area, hence in reduced flavour release (50). Thickness was lower in subjects with high α -amylase activities for the starch-based custard. This observation was not surprising, since α -amylase breaks the starch down, lowering the viscosity. There have been a number of studies showing that there is a strong relation between viscosity and perceived thickness (51-53). Slippery lip-tooth feel was stronger in subjects with low α -amylase activities. Conceivably the same mechanisms is at work as for protein concentration. High individual α -amylase activity also decreased creamy after feel in both custard and mayonnaise. One possible explanation for this is that the creamy after feel is derived from a coating consisting of a layer of starch particles attached to mucosa. As α -amylase hydrolyzes starch, breaking down the long starch strands up into smaller units, a high enzyme activity breaks down starch faster and more efficient while the product is still in the oral cavity. Consequently, this could reduce the amount of starch left available to form a coating. Possibly, this results in a thinner coating, if any. Probably not only the quality of a coating is of importance for the perception, but also the thickness. A conjecture is that a thicker coating results in a stronger creamy sensation. The reduced creamy sensation could also be related to the reduced flavour release (38). Findings from an unpublished study (de Wijk et al., in preparation) confirm the effect of α -amylase on creamy after feel. In that study an increase of creamy after feel was achieved by adding an amylase inhibitor to the custard.

In conclusion, the salivary components measured in this study varied considerably among subjects, but also within subjects as a result of different means of stimulation. These variations in salivary components were reflected in sensory perception of a number of flavour, mouth feel and after feel attributes in the semi-solids mayonnaise and custard dessert. Total protein concentration and α -amylase activity were observed to be correlated to the largest effects on texture perception. As saliva contains a multitude of different proteins, further studies on the effects and actions of the types of proteins is needed for a deeper understanding of the implications and applications of saliva composition in perception and acceptance of food.

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CHAPTER 13

DISCUSSION AND CONCLUSIONS

INTRODUCTION

The comprehensive results have been communicated in the previous chapters of this thesis. In this chapter a summary of the main results is presented, followed by a final discussion, in which the results are integrated. The chapter is concluded with suggestions for further research. For the convenience of the reader, the aim and background of this study are recapitulated from chapter 1:

The aim of this research was to examine the role of oral physiology on oral texture perception and investigate whether individual differences in perception could be attributed to and explained by differences in oral physiology among subjects.

Texture is an important descriptor of food products. Previous research on food texture has focused mainly on rheological measurements, frequently correlated with sensory data of the same product (introduction). Conventional rheological measurements are not based on oral systems and have often turned out to be unsatisfactory in explaining the relationship between food structure and texture perception. This could be explained by the notion that this approach disregards the oral processing and physiology of the mouth. Moreover, most sensations associated with food texture occur only when the food is manipulated, deformed, or moved across the oral receptors. During the time in the mouth, the stimulus undergoes constant changes: it is heated or cooled; diluted and broken down by saliva; and manipulated mechanically. This makes the mouth a very challenging system to mimic *in vitro*.

Another indication to that oral processes are important, is that human volunteers (subjects) assessing the same stimulus do not only differ largely in their ratings of that stimulus, the oral physiological parameters also exhibit large inter-individual variations. In this light, this research has focused on oral physiology as the “missing link” in understanding the relationship between food structure and texture perception.

The research was divided into four different topics (fig 6, chapter 1): Oral sensitivity and particles; manipulations of tongue movements; oral and product temperature; and saliva.

SUMMARY OF RESULTS

Oral sensitivity and particles

Semi-solids, here the stimulus, can be perceived as grainy, due to the fact that particles are sensed. The question is then whether the particles are sensed as part of the stimulus, or separately. To investigate the mechanisms of particles sensed separately, it was chosen to take things to extremes and an experimental set-up was chosen for the first two studies, in which spheres were used which could be handled separately and safely. In the two subsequent studies, we used particles of sizes naturally present in e.g. custard desserts.

Sphere size

The sizes of spheres ranging from 4 to 9 mm of different materials were assessed. The results revealed that size itself determines the size perception and that material and weight are negligible factors. We found an illusional effect in the direction of under-estimation for the spheres. This was especially true for the small sizes, up to 8 mm diameter. While covering the palate with a plastic plate, a significant improvement ($p < 0.05$) was observed; the subjects performed better and less under-estimation was observed (chapter 2). An explanation for this could be that only a minor part of the total area of the sphere touches the palate and is hence detected, while the tongue alone is more compliant and thereby able to sense the whole size of the sphere. This study thus suggests that oral size perception results from a combination of sensory inputs from the palate and the tongue.

Oral sensitivity

The accuracy of solid object size perception, spatial acuity and food particle size reduction during mastication were measured with/without topical anaesthesia of the oral mucosa (chapter 3). The sizes of food particles, chewed for 15 cycles and at swallowing, were found to correlate with individuals' ability to perceive the size of steel spheres (\varnothing 4-9 mm) between the tongue and palate, but not with their spatial sensitivity (measured by two-point discrimination). In contrast, topical anaesthesia had no effect on the perception of sphere sizes but significantly reduced spatial sensitivity. We suggest that the stimuli used to test two-point discrimination mainly stimulates superficial receptors, which involve light touch and are easily anaesthetized, while the spheres might excite more deeply-set receptors. The latter appear more important for masticatory performance and swallowing. Poor chewers seem to be sensitive to spheres in sizes that could cause them discomfort during swallowing.

Particles and size perception

Size and type of particle is important for size perception (chapter 4). Subjects are well able to perceive differences in particles sizes (2-200 μm) by rubbing a sample of particles dispersed in a medium between the tongue and palate. Since those particles are too small to separately activate pressure sensors with a confined sensory field, the suggested mechanism of size perception of particles is vibration (1). The hard and irregular silica particles are perceived as larger than soft and round polystyrene particles of the same size. Two different methods (direct scaling and forced ranking) of size assessment were compared. The results demonstrate that both methods provide very similar outcomes for studies on oral size perception. For the larger spheres (chapter 2), the tongue and palate signalled conflicting information. However, the topical anaesthesia did not affect size perception of small particles, possibly because also in this case, more deeply-set receptors were employed.

Particles and texture

Vibration is also suggested to be the mechanism driving the perception of roughness (chapter 5). Particle size affected rough sensations, and the strongest sensation was observed for particles around 80-140 μm . Again, silica had a stronger effect than polystyrene particles. In

soft products such as dairy desserts, roughness is not a very favourable attribute. The presumably more favourable attributes; slippery, creamy, smooth and fatty, were negatively influenced by the addition of particles, irrespective of particle type and size. This can possibly be explained by that other mechanisms, in addition to vibration, elicit the perception of these seemingly complex attributes. The mouth was observed to be highly sensitive to very small particles as demonstrated by the strong effect on smooth and rough sensations exerted by silica particles as small as 2 μm .

Roughness was negatively correlated with size perception of one certain size, indicating that beyond a certain particle size, even if the particles are strongly sensed and present, subjects no longer include the sensation of the particles in their assessment of rough perception. This suggests that in order for particles to have an effect on texture perception, it is important that they are sufficiently small.

Movements of the tongue

When placed in the mouth, food is subjected to an elaborate sequence of manipulations by the lips, teeth, cheeks, tongue and palate, and mixed with saliva, during which it is transformed in to a form suitable for swallowing. Sensations of the food attributes are gathered during each phase of the mastication process. To gain insight into the effect of oral processes on perception, we defined a set of five specific oral manipulations and investigated their effects on the perception of two types of semi-solids, vanilla custard desserts and mayonnaises (chapter 6). The oral manipulations ranged from simply placing the stimulus on the tip of the tongue to vigorously moving it around in the mouth. Most attributes showed a similar pattern, with lowest attribute ratings where the tongue's movement was restricted and gradually increasing ratings with increasing complexity of the tongue movements. An individual's normal mastication behaviour, which exhibited the largest diversity and complexity of movements, typically resulted in the most intense sensations of flavour and mouth-feel. This suggests that consumers aim to maximize their food sensations. Obviously, foods that are not liked should result in mastication behaviours aimed to minimize food sensations.

Results also indicate that the sensation of virtually all flavour and mouth-feel attributes requires at least some tongue movement. These manipulations mix food with saliva and thereby enhance mechanical and chemical breakdown, as well as position the food relative to the sense organs. The attributes least affected by tongue movements were typically those rated soonest after ingestion, in other words those that require no or only a few tongue movements to assess. The specific tongue movements required by the mouth-feel attributes provide information on the underlying mechanisms.

Temperature

Humans have a strong preference for the temperature of the products they consume (2;3). Different products exhibit diverse preferred intake temperatures; ice-cream is considered most

pleasant when eaten cold and French-fries taste the best when warm. Other products are consumed at different temperatures, depending on the context and culture.

Temperature affects the physical chemical properties of food. It follows from Jowitt's definition (4) that physical properties in turn influence texture: "Texture is the attribute of a substance resulting from a combination of physical properties and perceived by the senses of touch (including kinesthesia and mouthfeel), sight, and hearing". Hence, a change in physical property of food would influence the texture sensation. For instance, an increase in product temperature is known to change the viscosity, cause melting of fats and enhance flavour and odour release. The present study investigated the effect of oral and product temperature on temperature perception and on the resulting texture attributes of semi-solids.

Perception of temperature

The first thermal study (chapter 7) examined the influence of oral temperature on oral perception of temperature in liquids and semi-solids. Subject assessed the temperature of water, custard dessert and mayonnaise. Oral temperatures were manipulated prior to assessments, which resulted in oral temperatures of 27 °C, 35°C and 43°C, respectively. The products were evaluated at 10°C, 22°C and 35°C. Results demonstrate that subjects were able to differentiate between the product temperatures. A large effect of type of product was seen on perceived temperature, where water was over-all perceived as significantly colder than custard dessert and mayonnaise. The range of perceived thermal ratings was widest for custard dessert, followed by water and mayonnaise. This might be due to differences in composition and structure of the products. Even though oral temperature was varied considerably in the present study, this did not exert large effects on perceived temperature.

Temperature and texture perception

In the second thermal study (chapter 8), we sought to study the effect of oral and product temperature on the perception of texture and flavour attributes. The subjects assessed texture and flavour attributes of two semi-solids: custard dessert and mayonnaise. The products were evaluated at different temperatures (see chapter 7). Results showed that modulation of product and oral temperature had significant effects on a number of attributes. Flavour intensities, melting mouth feel, and fat after feel increased, while subjective thickness decreased with increasing product temperature. Neither product- nor oral temperature had an effect on over-all creaminess. Oral temperature affected a number of mouth feel attributes: e.g. melting and heterogeneous, where a high oral temperature increased both perceptions. A possible explanation could be an increased enzymatic action, leading to more structure breakdown. The high temperature itself could cause a change in viscosity of the stimulus in the mouth and hence a more melting sensation. This decrease in viscosity at the surface of the stimulus, in comparison to the bulk, could lead to a heterogeneous sensation when advancing the tongue through the stimulus.

From this study it can be concluded that product and oral temperature influence the perception of certain flavour and texture attributes in semi-solids, with a larger effect on

custard dessert than on mayonnaise. For the evaluation of food products, it is therefore important to consider if the food is going to be eaten in combination with e.g. a hot or a cold drink, as this might influence the perception of the product. The fact that variation of product and/or oral temperatures highlights specific flavour/texture sensations, may be useful for product development or quality control where one typically wants to focus on certain sensations and ignore others.

Saliva

Saliva is always present in the mouth, where it serves many functions. The amount and composition of saliva, however, varies with the state (sleep/awake relaxed/stressed) of the individual (5). During eating, the amount of saliva increases and the degree depends on the type of stimulation. Saliva consists for 99% of water, and in the remaining 1 % there is a multitude of other components, such as ions, enzymes and other proteins. This results in that tastants dissolve in saliva and are therefore more easily transported to the taste buds, enabling us to taste the food (6-8). In addition, the food is mixed, diluted and broken down by saliva, which changes the texture of the food. Mixing the food with saliva, including breakdown and dilution, is considered to be of large importance for semi-solids as these products are masticated without chewing. Consequently, saliva may also have an effect on the way we perceive texture. Between meals, saliva wettens and protects the mucosa and the teeth, and it has anti-bacterial and anti-inflammatory properties (9;9-12).

Chewing behaviour

In a first study on saliva (chapter 9), we investigated the influence of salivary flow rate on the chewing process. Widely different types of food were included in the study; dry and crisp melba toast, sweet and moist cake and fat cheese. We observed a larger number of chewing cycles until swallowing for foods with less water and fat. A dry product needed a longer time in the mouth to allow for enough secretion of saliva for the formation of a food bolus that can be swallowed, because the salivary flow rate (ml/min) did not increase for a product with less water and fat.

Salivary flow rate and addition of fluids

Two studies on the effect of saliva volume on texture perception were performed. In order to investigate if the individual salivary flow rate influences the sensory ratings, salivary flow rates at rest and after three types of stimulation were correlated with sensory ratings of vanilla custard dessert (chapter 10). No significant correlations could be found between any of the salivary flow rates and the sensory ratings. This finding could indicate that subjects are used to their respective amounts of saliva to such a degree that the differences in sensory ratings between subjects cannot be explained by the inter-individual difference in saliva flow rate.

The effect of adding saliva or a saliva-related fluid (α -amylase solution and water) to custard dessert prior to ingestion on sensory perceptions was investigated (chapter 11). The results show that the addition of a fluid mainly affected attributes related to viscosity. The effect was volume dependant, where a larger volume enhanced the effect. Melting was the only attribute

on which the type of fluid had an effect, where saliva elicited a stronger melting effect than the α -amylase solution and water.

Composition of saliva

As it is known that there are large variations in composition of saliva originating from different glands and different subjects (13), our next step was to investigate how variations in salivary characteristics affect sensory perception (chapter 12). Variations in salivary components were correlated with sensory perception of a number of sensory attributes of mayonnaise and custard dessert. Total protein concentration and α -amylase activity were observed to correlate most strongly with texture perception.

FINAL DISCUSSION

The aim of the study was to examine the role of oral physiology on texture perception of semi-solids. In this quest, I have covered a number of widely different oral physiological parameters in relation to texture perception of semi-solids, which are reported in this thesis.

Semi-solids was confirmed to be a practical stimulus group to work with, due to the ease with which they could be modified in a quite reproducible manner, to produce a large variation of stimuli. In addition, the exclusion of the chewing process enabled me to focus on other mechanisms and structures in the mouth than the teeth, which is a quite unexplored area. Flavour and taste have been demonstrated to influence texture perception (14). Therefore we chose to keep the taste and flavour constant as far as possible in all studies presented in this thesis.

One of the main observations is that there is a large inter-individual variation for most, if not all of the oral physiological parameters. This was a confirmation of the expectations. The interesting finding is that these differences in many cases correlate with the individual perception of semi-solids.

Oral sensitivity and size perception

The sensitivity of the tongue and palate is extraordinary in comparison to most other parts of the body. These studies accordingly showed that there is evidence pointing towards the relation between oral sensitivity and oral perception. In chapter 5, the interesting finding was made that subjects who rated particles of a certain size in a stimulus to be quite large, did not find that same stimulus to be very rough. Hence, for particles to contribute to the perception of roughness, they need to be small enough, or else they are not considered to be part of the stimulus. Not only the actual oral sensitivity affects the perception of size; poor chewers, e.g., have been observed to be more sensitive to and over-estimate particles in sizes that might be uncomfortable for them to swallow.

Perceived size of spheres in the mouth was dependent on the actual size of the sphere and not on the weight. This suggests that the size of the object is derived from the number and spatial arrangement of stimulated receptors, and not on the magnitude of the pressure exerted by the object.

Palatal coverage improved the subjects' size assessment by reducing the underestimations of small sphere sizes. It may sound a bit controversial that shutting out receptors would lead to a better outcome. However, this indication is probably a result of the object's shape, since tongue and palate did not have the same area of contact with the sphere and hence signalled conflicting information on the size. On the contrary, in an unpublished study where texture attributes were rated with and without palatal coverage, we found that the intensity of some attributes decreased with a palatal coverage, while others remained unaffected. Most likely the employment of the entire mouth results in the strongest intensity. Studying the response of different oral parts to stimuli can provide information on the mechanisms behind oral sensation and perception.

Saliva

The natural volume of saliva did in the case of our products not affect the texture perceptions, presumably because the subjects were used to their inherent levels of saliva. In contrast, a change to the natural amounts of saliva, realized by an artificial addition of saliva or saliva related fluids, did have some effects on attributes related to dilution and breakdown of the product, such as thickness and melting.

The composition of saliva, however, exhibited correlations with the perception of numerous texture attributes. These results suggest that for semi-solids, in contrast to proteins and solubles, the water part of saliva is not an important determinant for perception. A conjecture is that since semi-solids are naturally moist and soft, the water of saliva is not necessary to wetten the stimulus, to form a bolus, or to release taste and flavour compounds. For dryer products, however, saliva can be important for the perception, as a dry product was chewed for a longer time before being suitable to swallow. The fractions of solubles in the saliva, many of which have enzymatic, lubricative or other actions important for mastication, do seem to be important for the perception of semi-solids. Many attributes are dynamic, hence their perception is based on changes monitored in the mouth. One can imagine that a higher activity of enzymes could affect the perception, due to a rapid breakdown of the stimulus. Accordingly, the attributes for custard dessert, which is thickened mainly by starch, were especially sensitive to the activity of amylase. This has also been shown in studies where the activity was varied and where the enzymatic action was blocked (patent #). Amylase hence seems to be very important for the attributes thickness, melting, creamy and fatty.

Stimuli modifications

Stimuli modifications were in many cases very potent in affecting the texture perception. The primary result of an increase in stimulus temperature probably was the decrease in stimulus

viscosity, which was reflected in decreased perceptions of thickness. With the decrease in viscosity, a number of secondary effects were observed, such as facilitated migration of fat to the surface of the product, resulting in more fatty sensations.

The addition of particles to the stimulus also resulted in large changes in perception of many attributes. It is suggested that the mechanisms with which particles affect the attributes is vibration, possibly registered by FA I receptors in the tissue. Katz argued that two sorts of cues contribute to skin texture perception: spatial cues for coarse surfaces, and vibrational cues for fine surfaces (1). For finer surfaces, whose excursions into the third dimension (bumps or gaps) are too small to be resolved by the mechanoreceptors, Katz proposed that texture encoding depends primarily on microscopic vibrations set up in the skin as the stimuli is rubbed along it. Hollins et al. (15) provided evidence for this idea by comparing the subjective smoothness between a stationary surface and a surface that was vibrated at different frequencies (150-400Hz). The vibrating surface was judged as less smooth. Evidence supporting this, is that in stimuli with a higher fat level, or when fat was added as a coating, fat acted as a lubricant and perceptions of roughness decreased (16).

CONCLUDING REMARKS

In this thesis, the first part of the aim concerned the role oral physiology plays in oral texture perception. The results demonstrate that the oral physiological parameters oral sensitivity, tongue movements, temperature and saliva composition are of importance for texture perception of semi-solids. The second part of the aim of the study addressed whether individual differences in perception could be attributed to variations in oral physiology. A number of significant correlations confirm that oral physiology could explain some of the intra-individual variation. These results indicate that oral physiology is an important aspect and should not be neglected in texture research. Nevertheless, the studied parameters do not provide the entire solution. An explanation for this could be that there are probably more “missing links”. Oral physiology deals with the conditions around the peripheral receptors. However, the signals from peripheral receptors are conveyed to the central nervous system, where they are integrated, resulting in the perception. Besides physiological differences, which are partly genetic, differences in texture perception could be attributed to environmental factors such as social context and culture.

SUGGESTIONS FOR FUTURE RESEARCH

In recent years the interest in texture has expanded immensely. Still, as research is performed, the results lead to new questions. This study has triggered a whole range of questions, which could all be potential future research topics.

First, all the studies reported in this thesis have been performed in healthy subjects. To be able to test some of the hypotheses and mechanisms, it would be interesting to compare healthy and pathological conditions. A couple of suggestions include patients with xerostomia or decreased tongue mobility.

Secondly, a typical sensory profile of custard dessert indicates that rough/astringent is opposite the smooth/creamy group of attributes. This and the results of some of the studies here suggest that there is a rough – smooth continuum, which is characterized by the presence or absence of particles or astringents. As these attributes are major components for the appreciation of semi-solids, this would be an interesting subject to investigate further.

Salivary components were demonstrated to correlate with the perception of a number of texture attributes and this was especially true for α -amylase and proteins. “Total protein” is however a large group of different proteins (more than 40 salivary proteins have been identified (17)) and for a better understanding of the mechanisms of texture attributes, it would be interesting to separate the effect of the different proteins on the stimuli and hence on texture perception.

Some texture attributes of semi-solids may be related mainly to the bulk properties of the stimulus, such as thickness or heterogeneity, whereas others, e.g. roughness and dryness may be based on mainly on the stimulus’ surface properties (Unpublished data de Wijk *et al.*). More insight into these bulk or surface properties could prove valuable for the understanding of the texture attributes and provide indications to appropriate instrumental measurements.

We have tried to keep the stimuli as constant as possible regarding odour and flavour in some of the studies. Nonetheless, research in this laboratory has suggested that interaction between flavour and texture often takes place (14). In that respect, further investigation into the variations and interactions between texture and flavour and odour would be motivated. Another possibility is to control aspects, such as bite size and oral residence time.

Many aspects of texture are most likely perceived as mechanical stimuli. In order to fully understand the mechanisms underlying texture attributes, there is a need to characterize which mechanoreceptors give rise to what sensations. This could be done by using electrophysiological techniques. As mechanoreceptors in the mouth are not yet fully understood, a deeper insight into these receptors and their exact function in food sensation would be of great importance in this area of research.

Sensations originate from peripheral receptors. Perception however, is the result of signal integration in the brain. Often there are multimodal interactions, where integration between the senses takes place. Rolls *et al.* have investigated multimodal neurons in orbitofrontal cortex of primates by single cell recording (18). They have found that signals of vision, taste, olfaction and touch converge in the orbitofrontal cortex. Many neurons respond to a combination of these, e.g. viscosity, taste and irritation (19;20). One does also wonder what “hard ware” in the brain separates out the different types of texture. In human, less invasive methods are preferred, where MRI e.g. might play a greater role in the future. Further research into neurons responding to different types of texture would be interesting.

Further suggestions are based on fig 2 (chapter 1). In this study, we have not focused on extra-oral cues and their effect on texture perception.

A more psychological approach to texture perception could include a comparison between culture, experience, mood and expectations.

The physiological parameters studied in this thesis are local to the oral region. Texture perception could also be related to more systemic physiology, such as body hydration, metabolism and circadian variations.

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CHAPTER 14

SUMMARY

WITH DUTCH AND SWEDISH TRANSLATIONS

SUMMARY

We eat several times a day and most of the time we are actively aware of what we eat. The food we eat undergoes many events on its way from the plate to the stomach. In the oral cavity, the food is subjected to several mechanical and chemical processes. It is diluted and broken down by saliva, heated or cooled by the ambient temperature of the mouth, formed into a bolus and finally swallowed. The numerous receptors in the oral cavity and nose respond to the initially ingested food and monitor the changes during processing. This leads to central perceptions of taste, odour, irritation and texture of the food.

Texture is an important descriptor of food products and is defined as: “Texture is the attribute of a substance resulting from a combination of physical properties and perceived by the senses of touch (including kinesthesia and mouthfeel), sight, and hearing. Physical properties may include size, shape, number, nature and conformation of constituent structural elements”¹.

Previous research on food texture has focused mainly on rheological measurements, frequently correlated with sensory data of the same food. However, conventional rheological measurements are not based on the oral system. Most sensations associated with food texture occur only when the food is manipulated, deformed, or moved across the oral receptors. In addition, people assessing the same stimulus differ in their ratings of that stimulus and their oral physiological parameters also exhibit inter-individual variations. In this light, oral physiology is thought to be the “missing link” in understanding the relationship between food structure and texture perception.

The aim of this research was to improve the understanding of oral texture perception. In particular to examine the role of oral physiological processes on oral texture perception of semi-solids and to investigate whether individual differences in perception could be attributed to differences in oral physiology among subjects.

The research was divided into four different topics (fig 6, chapter 1): Oral sensitivity and particles; manipulations of tongue movements; oral and product temperature; and saliva. A summary of the separate studies is presented in chapter 13.

The approach to investigate the effect of oral physiology was as follows:

1. Individual parameters of oral physiology were studied. These include tongue movements and different characteristics of saliva, oral sensitivity to temperature and to particle sizes ranging from 2 μm to 9 mm.

¹ Jowitt R. The terminology of food texture. *J Text Stud* 1974; 5:351-358.

2. Physiological parameters were artificially altered, such as the addition of extra saliva or modification of oral temperature and tongue movements.
3. Stimuli were modified by changing the temperature, or by adding particles or fluid.
4. Sensory tests were performed on trained subjects for both unmodified and modified stimuli (as in 3) and for artificially altered physiological parameters (as in 2).
5. Sensory data was correlated with individual physiological parameters (4 and 1).

A combination of two or more of these methods was applied in each study. The main results of the research are presented below. These reveal that:

- Large inter-individual differences in oral physiology and sensory ratings were confirmed.
- Oral size perception results from a combination of sensory inputs from the palate and the tongue. Size itself determines the size perception of single objects, while material and weight are negligible factors.
- For particles mixed into a medium, size and type of particle influenced size perception, which is suggested to be mediated by vibration. Soft and round particles are perceived as smaller than hard and irregular particles.
- The two methods - direct-scaling and forced ranking - produce similar results on size perception.
- Some measurements of oral sensitivity correlate, such as masticatory performance and size perception of spheres. Subjects who are poor chewers are more sensitive to particles in sizes which could cause then discomfort on swallowing.
- The natural volume of saliva did not affect the texture perceptions, presumably because the subjects were used to their inherent levels of saliva. In contrast, a change in the natural amounts of saliva, realized by an artificial addition of saliva or saliva related fluids, did affect attributes related to dilution and breakdown of the product, such as thickness and melting.
- The composition of saliva exhibited correlations with the perception of numerous texture attributes. The fractions of solubles in the saliva, many of which have enzymatic, lubricative or other actions important for mastication, do seem to be important for the perception of semi-solids. Accordingly, the attributes for custard dessert, which is thickened mainly by starch, were especially sensitive to the activity of amylase and proteins.
- The sensation of virtually all flavour and mouth-feel attributes requires at least some tongue movement. Most attributes showed a similar pattern, with lowest attribute ratings where the tongue's movement was restricted and gradually increasing ratings with increasing complexity of the tongue movements. An individual's normal

mastication behaviour, which exhibited the largest diversity and complexity of movements, typically resulted in the most intense sensations of flavour and mouth-feel. This suggests that consumers aim to maximize their food sensations.

- The addition of particles to a custard dessert had large effects on perception of the texture attributes. Roughness increased with increasing particle size and hardness, while smooth, slippery, creamy and fatty decreased, irrespective of type of particle. Very small particles (2 μm) also have strong effects on the perception. Subjects who perceive particles of a certain size to be large, do not perceive that same stimulus to be rough. Hence, particles need to be sufficiently small to be considered part of the stimulus.
- The primary result of an increase in stimulus temperature probably was the decrease in stimulus viscosity, which was reflected in decreased perception of thickness. With the decrease in viscosity, a number of secondary effects were observed, such as facilitated migration of fat to the surface of the product, resulting in more fatty sensations.

The results demonstrate that the oral physiological parameters oral sensitivity, tongue movements, temperature and saliva composition are of importance for texture perception of semi-solids. Many parameters of oral physiology correlated with various texture attributes. This implies that inter-individual differences in texture perception could be attributed to variations in oral physiology. Oral physiology thus plays a role in texture perception of semi-solids and should be taken into account in future texture research.

SAMENVATTING

Wij eten meerdere malen per dag en zijn ons dan meestal actief bewust van wat we eten. Op de weg van het bord naar de maag ondergaat het voedsel vele bewerkingen. In de mondholte vinden mechanische en chemische processen plaats. Het voedsel wordt verdund en afgebroken door het speeksel, opgewarmd of afgekoeld door de temperatuur van de mond, vervormd tot een bolus en uiteindelijk doorgeslikt. De vele receptoren in de mondholte en de neus reageren op het voedsel en zij detecteren de veranderingen die het voedsel ondergaat. Dit leidt tot de centrale perceptie van smaak, geur, irritatie en textuur van het voedsel.

Textuur is een belangrijke eigenschap van levensmiddelen. Het wordt als volgt gedefinieerd: “Textuur is het kenmerk van een substantie en het is het resultaat van een combinatie van fysische eigenschappen en van de waarneming door de zintuigen tast (inclusief kinestese en mondgevoel), zicht en gehoor. De fysische eigenschappen omvatten o.a. grootte, vorm, aantal, aard en configuratie van de structurele elementen¹.

Eerder textuuronderzoek was voornamelijk gericht op rheologische metingen, veelal gecorreleerd aan sensorische gegevens van hetzelfde voedsel. Echter, conventionele rheologische metingen hebben weinig van doen met wat er in het orale systeem plaats vindt. De meeste waarnemingen, die geassocieerd worden met textuur van voedsel, vinden juist plaats wanneer het voedsel gemanipuleerd en vervormd wordt in de mond en langs de orale receptoren beweegt. Het blijkt, dat mensen die hetzelfde voedsel beoordelen, verschillen in hun waardering van dat voedsel. Tevens blijkt dat de oraalfysiologische eigenschappen verschillen van persoon tot persoon. Orale fysiologie wordt daarom mogelijk gezien als de ontbrekende schakel in het begrijpen van de relatie tussen de structuur van het voedsel en de textuurperceptie van dat voedsel.

Het doel van dit onderzoek was om de kennis van orale textuurperceptie te vergroten. Met name werd de rol van oraalfysiologische processen op orale textuurperceptie van zacht voedsel bestudeerd. Verder werd onderzocht of individuele verschillen in perceptie verklaard konden worden door verschillen in orale fysiologie tussen personen. Het onderzoek werd onderverdeeld in vier onderwerpen (zie hoofdstuk 1, figuur 6): 1. orale sensitiviteit en kleine voedseldeeltjes, 2. manipulatie van tongbewegingen, 3. mond- en producttemperatuur, 4. speeksel. Een samenvatting van de verschillende studies werd in hoofdstuk 13 gepresenteerd.

¹ Jowitt R. The terminology of food texture. *J Text Stud* 1974; 5:351-358.

Het effect van orale fysiologie werd als volgt bestudeerd:

1. Individuele parameters van de orale fysiologie werden bestudeerd. Dit omvat tongbewegingen, verschillende eigenschappen van speeksel, orale sensitiviteit voor temperatuur en voor korrelgroottes variërend van 2 μm tot 9 mm.
2. Fysiologische parameters werden kunstmatig veranderd, bijvoorbeeld door het toevoegen van extra speeksel of door het aanpassen van mondtemperatuur en tongbewegingen.
3. Stimuli werden gemodificeerd door de temperatuur te veranderen of door het toevoegen van korrels of vloeistof.
4. Sensorische testen werden uitgevoerd bij getrainde proefpersonen met zowel niet-gemodificeerde als gemodificeerde stimuli (punt 3) en met kunstmatig veranderde fysiologische parameters (punt 2).
5. Sensorische gegevens werden gecorreleerd met individuele fysiologische parameters (punt 4 en 1).

In elke studie werd een combinatie van twee of meer van deze methoden toegepast. Hieronder volgt een overzicht van de belangrijkste resultaten:

- De eerder gevonden grote verschillen tussen proefpersonen in orale fysiologie en sensorische beoordelingen werden bevestigd.
- Orale perceptie van deeltjesgrootte is het resultaat van een combinatie van sensorische input van het gehemelte en de tong. De grootte van de deeltjes zelf bepaalt de grootteperceptie van die deeltjes, terwijl materiaal en gewicht niet belangrijk bleken te zijn voor grootteperceptie.
- Grootte en soort van deeltjes, die door een substantie worden gemengd, beïnvloeden de perceptie van de grootte. Dit wordt waarschijnlijk tot stand gebracht door vibratie. Zachte en ronde deeltjes worden kleiner waargenomen dan harde en onregelmatig gevormde deeltjes.
- Twee methodes – directe schaling en geforceerde rangschikking – leveren vergelijkbare resultaten op voor de perceptie van de grootte van deeltjes.
- De resultaten van sommige metingen van de orale sensitiviteit zijn significant gecorreleerd, zoals bijvoorbeeld van kauwvermogen en grootteperceptie van bolletjes. Proefpersonen met een slecht kauwvermogen zijn gevoeliger voor deeltjes met afmetingen die ongemak zouden kunnen veroorzaken bij het doorslikken.
- Het natuurlijke volume van het speeksel van een persoon heeft geen invloed op de perceptie van textuur. Dit komt waarschijnlijk, omdat een persoon gewend is aan zijn

eigen hoeveelheid speeksel. Een verandering in de natuurlijke hoeveelheid speeksel daarentegen, gerealiseerd door kunstmatig toevoegen van speeksel of van op speeksel gelijkende vloeistoffen, had wel effect op bepaalde kenmerken van het voedsel. Deze kenmerken waren gerelateerd aan verdunning en afbraak van het product, zoals bijvoorbeeld dikte en smelten.

- De samenstelling van speeksel vertoont correlatie met de perceptie van diverse textuurkenmerken. Stoffen in het speeksel, waarvan vele een enzymatische, smerende of andere werking hebben, die nodig zijn voor het kauwen, lijken belangrijk te zijn voor de perceptie van zacht voedsel. Derhalve zijn de attributen van vla, die voornamelijk verdikt zijn door zetmeel, speciaal gevoelig voor de activiteit van amylase en eiwit.
- Voor de gewaarwording van vrijwel alle smaak en mondgevoelattributen is enige tongbeweging noodzakelijk. De meeste attributen vertonen een vergelijkbaar patroon, met de laagste attribuutwaarden voor de meest beperkte tongbeweging. De attribuutwaarden namen toe voor complexere tongbewegingen. De hoogste waarden voor smaak en mondgevoel werden verkregen tijdens normaal kauwen. De tongbeweging is dan het meest complex. Dit zou kunnen betekenen, dat consumenten hun voedselgewaarwording zo maximaal mogelijk proberen te maken.
- De toevoeging van deeltjes aan vla had grote effecten op de perceptie van textuur attributen. De ruwheid nam toe bij toenemende deeltjesgrootte en hardheid, terwijl glad, glibberig, romig en vettig afnamen, onafhankelijk van het type deeltjes. Heel kleine deeltjes (2 μ m) hebben ook reeds een sterk effect op de perceptie. Personen, die deeltjes van een bepaalde grootte daadwerkelijk waarnemen als deeltjes, ervaren de stimulus niet langer meer als ruw. Dus deeltjes moeten klein genoeg zijn om ze als onderdeel van de stimulus te kunnen beschouwen.
- Het voornaamste resultaat van een toename in de stimulus temperatuur was waarschijnlijk de afname in stimulusviscositeit. Dit vertaalde zich in een afname van de dikteperceptie. Met de afname in viscositeit werd een aantal afgeleide effecten waargenomen, zoals een makkelijker verlopend transport van het vet naar de oppervlakte van het product. Dit had als resultaat, dat het product als vetter werd beoordeeld.

De resultaten tonen aan dat de oralfysiologische parameters, zoals orale gevoeligheid, tongbeweging, temperatuur en speekselsamenstelling van belang zijn voor textuurperceptie van zacht voedsel. Vele parameters van de orale fysiologie correleren met diverse textuur attributen. Dit betekent dat inter-individuele verschillen in textuurperceptie kunnen worden toegeschreven aan variaties in de orale fysiologie. Orale fysiologie speelt dus een belangrijke rol in textuurperceptie van zacht voedsel. Daarom zal in toekomstig onderzoek naar textuur rekening moeten worden gehouden met de orale fysiologie.

SAMMANFATTNING

Vi äter flera gånger om dagen och för det mesta är vi medvetna om vad vi äter. Maten vi äter går igenom många processer på sin väg från tallrik till mage. I munhålan blir maten utsatt för åtskilliga mekaniska och kemiska processer. Maten förtunnas och bryts ned av vår saliv, värms eller kyls ned av munnens temperatur, formas till en sväljbar bolus och sväljs.

De många receptorererna i munhålan och näsan känner av den mat som vi intar och registrerar ändringarna under processen. Det här leder till centrala perceptioner av matens smak, lukt, irritation och textur.

Textur är en viktig beskrivning av matprodukter och definieras som följer: ”Textur är ett substansattribut, som är resultatet av en kombination av fysiska egenskaper och av varseblivning genom sinnen; beröring (kinesi och munkänsla), syn och hörsel. Fysiska egenskaper kan vara storlek, form, antal, karaktär och konfirmation av sammansatta, strukturella element¹.

Tidigare forskning på mattextur har framförallt fokuserats på reologiska mätningar, som ofta är korrelerade med sensoriska data av samma matprodukt. Emellertid är inte konventionella reologiska mätningar baserade på det orala systemet. De flesta sensationer (upplevelser), som associeras med mattextur uppstår endast när maten är manipulerad, deformerad eller förs över receptorerna. Dessutom ger personer som bedömer samma stimulus olika gradering och deras oralfysiologiska parametrar visar stora individuella skillnader.

Med utgångspunkt från detta tros oralfysiologi vara den saknade länken för förståelse av relationen mellan matstruktur och texturperception.

Syftet med den här forskningen var att öka förståelsen av oral texturperception och framförallt undersöka oralfysiologiska processers inflytande på den orala texturperceptionen av semisolid produkter och utreda huruvida individuella skillnader i perceptionen kan tillskrivas de individuella skillnaderna i oralfysiologi.

Tillvägagångssätten att undersöka effekten av oralfysiologin var följande:

1. Individuella oralfysiologiska parametrar undersöktes. Dessa parametrar inkluderar tungans rörelser och salivens olika karakteristika, oral känslighet för temperatur och partikelstorlekar varierande från 2 µm till 9 mm.
2. De fysiologiska parametrarna ändrades artificiellt genom tillägg av extra saliv eller modifiering av oraltemperatur och orala rörelser.

¹ Jowitt R. The terminology of food texture. *J Text Stud* 1974; 5:351-358.

3. Stimuli modifierades genom temperaturändring, eller genom tillägg av partiklar eller vätska.
4. Sensoriska tester utfördes på tränade försökspersoner på omodifierade och modifierade stimuli och med artificiellt ändrade fysiologiska parametrar.
5. Sensoriska data korrelerades med individuella fysiologiska parametrar.

En kombination av två eller flera av dessa metoder användes i varje studie. De huvudsakliga forskningsresultaten presenteras här nedan:

- Stora individuella skillnader i oralfysiologi och sensorisk gradering konfirmerades.
- Oral storleksperception beror på en kombination av sensorisk input från palatum och tungan. Storleken i sig avgör storleksperceptionen av enskilda objekt, medan material och vikt är negligerbara faktorer.
- Storlek och typ av partikel påverkade storleksperceptionen för partiklar tillsatta till ett medium, vilken förmedlas genom vibration. Mjuka och runda partiklar uppfattades som mindre än hårda och oregelbundna.
- Två metoder – direktgradation och tvingad rangordning – ger liknande resultat på storleksperception.
- En del mätningar på oral känslighet, så som tugg effektivitet och storleksperception av partiklar, korrelerar. Försökspersoner som tuggar dåligt är känsligare för partiklar i storlekar som skulle kunna orsaka obehag vid sväljning.
- Den naturliga salivvolymen påverkade inte texturperceptionen, antagligen för att försökspersonerna var vana vid den medfödda nivån av saliv. En förändring av salivens naturliga mängd, genom artificiell addition av saliv eller salivrelaterade vätskor, påverkade däremot de attribut som är relaterade till förtunning och nedbrytning av produkten, såsom tjocklek och upplösning.
- Sammansättningen av saliv korrelerade med perceptionen av en mängd texturattribut. Lösliga fraktioner i saliven, av vilka många har enzymatisk eller smörjande funktion, verkar vara essentiella för perceptionen av semisolda produkter. Följaktligen var attributen för vaniljdessert, vilken fått sin konsistens huvudsakligen genom stärkelse, särskilt känsliga för amylas- och proteinaktivitet.

- Upplevelsen av in princip alla smak- och munkänslattribut fordrar åtminstone någon tungrörelse. De flesta attributen visade ett liknande mönster med lägsta attributgradering när tungans rörelser var begränsade och med stigande gradering vid ökande komplexitet av tungans rörelser. En individs normala tungbeteende, som visade den största variationen av och komplexiteten i rörelser, resulterade i de mest intensiva upplevelserna av smak och munkänsla.
- Tillägg av partiklar till en vaniljdessert hade stor effekt på perceptionen av texturattributen. Kornigheten ökade med större partiklar och hårdhet medan mjukhet, halhet, krämighet och oljeaktighet minskade oberoende av typ av partiklar. Även mycket små partiklar (2 μ m) påverkar perceptionen mycket. Försökspersoner vilka uppfattar en partikel i en särskild storlek som stor, upplevde inte samma stimulus som kornigt. Följaktligen måste partiklarna vara tillräckligt små för att uppfattas som en del av stimulus.
- Det primära resultatet av en ökning i temperaturen hos stimulus var förmodligen en minskning av stimulus viskositet, vilket återspeglades i minskad tjockleksperception. Vid minskningen av viskositet, observerades ett antal sekundära effekter, så som underlättande av transport av fett till produktens yta, vilket resulterade i en mer oljeaktig upplevelse.

Resultaten visar att de orala parametrarna; oral känslighet, tungrörelser, temperatur och salivens sammansättning är viktiga för texturperceptionen av semisolida stimuli. Många oral fysiologiska parametrar korrelerade med olika texturattribut. Detta implierar att individuella skillnader i texturperception kan hänföras till variationer i oralfysiologin. Oralfysiologi har därför betydelse för texturperceptionen av semisolida produkter och bör därför beaktas i framtida texturforskning.

CURRICULUM VITAE

Lina Engelen was born on May 19, 1974 in Trångsund, Sweden. In 1993 she graduated from the Erik Dahlbergsgymnasie in Jönköping. After one year administrative work in the Netherlands Antilles, she commenced a biology study at the University of Lund in Sweden specialising in physiology. During her studies, Lina studied one year at the Rijksuniversiteit Utrecht, the Netherlands as part of the Erasmus exchange programme. In 1998 she successfully finalised her masters degree with a research project on allergy, where plasma exudation in skin as a response to allergens was studied. Back in the Netherlands Antilles, she worked as a research assistant in two projects; one on marine biology and one related to haematology. Next she worked on a progress control and trial system at Rijkswaterstaat in the Netherlands. From September 2000 to May 2004, she was employed as a PhD-fellow at the oral physiology group at the University Medical Center Utrecht where she was seconded to the Wageningen Centre for Food Sciences. She is married to Peter.

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Travel – that’s a red thread through my existence, whether it’s treading the winding paths of science, the daily train journey to work, monthly trip to Holland while living in England,

regular family visit or the (at least) yearly vacation to an exciting place. Despite my efforts, I can't seem to keep up with my "places-I-want-to-see" list. Most of the time I love travelling; seeing new places, trying different food and meeting new people. This all, just as my life, begun with my parents.

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Lina