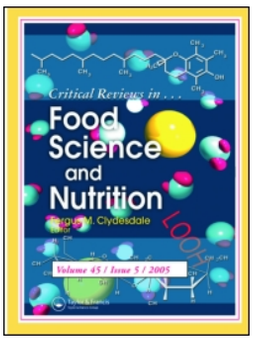

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Urška Pivk Kupirovič, Ibrahim Elmadfa, Marcel-Alexandre Juillerat & Peter Raspor (2017) Effect of saliva on physical food properties in fat texture perception, Critical Reviews in Food Science and Nutrition, 57:6, 1061-1077, DOI: 10.1080/10408398.2013.766787

To link to this article: http://dx.doi.org/10.1080/10408398.2013.766787

Accepted author version posted online: 12 May 2016.
Published online: 12 May 2016.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
2017, VOL. 57, NO. 6, 1061–1077
http://dx.doi.org/10.1080/10408398.2013.766787

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Effect of saliva on physical food properties in fat texture perception

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ABSTRACT

Sensory properties of food drive our food choices and it is generally accepted that lipids greatly contribute to the sensory properties of many foods and consequently to eating pleasure. Many studies have investigated the mechanisms of the fat perception. Unfortunately they used a variety of methods and products, thereby making generalization very difficult. The mechanism of fat perception in oral cavity is combined of several processes. Lipid composition and its properties strongly influence food structure. During consumption food is exposed to a range of in-mouth processing steps. Oral sensation of fat texture changes with time, from a first bite to chewing, while mixing with saliva, up to swallowing and even after swallowing. The present work reviews many aspects of fat texture perception from physical chemistry to physiology. Understanding the underlying mechanisms of in-mouth lipid processing would provide new concepts to produce low-fat food products with full-fat perception.

KEYWORDS

Lipids; in-mouth; texture; saliva; perception; interaction; food structure

Introduction

Sensory properties of food govern our food choices: lipids are responsible for the sensory properties of many foods and greatly contribute to eating pleasure. Despite attractive characteristics of lipids like palatability and high energy content, lipid consumption has been associated with higher rates of obesity and coronary heart disease. For this reason nutritional education efforts have focused on replacing dietary lipids with other foods such as, grains, vegetables, and fruit or by consuming foods which mimic sensory attributes of lipids with fat replacers (Akoh, 1995). Fat texture perception is defined in our review as a sensation given by fat or fat replacers in food.

Many studies have investigated the mechanisms of the fat perception (Table 1). The mechanism of fat perception in oral cavity is combined of several processes. Complexity of lipid composition and its properties tremendously influence food structure, which governs perceived fat texture. Fat texture has been correlated in liquid foods with viscosity as a structural parameter, which changes according to lipid content and quality, type of emulsifier or oil droplet size. However, for solid foods, fat perception is more complicated and we are easily mistaken in judging fat content (Drewnowski, 1997). Pleasantness and hedonic ratings of stimuli tend to increase with increasing lipid content (Drewnowski and Greenwood, 1983; Mela, 1988; Drewnowski et al., 1989). Food is exposed to a range of processing steps during consumption. It is broken down by chewing, mixed with saliva, heated or cooled to body temperature, air is introduced and it comes into contact with oral surfaces (Salles et al., 2010). Oral texture sensations of foods changes with time, from first bite to chewing, swallowing and after swallowing (Fig. 1) (van Vliet et al., 2009). Solid lipids may melt in the mouth and in the same way as oils lubricate the food during mastication and facilitate manipulation and swallowing. Some of the lipids, for instance free fatty acids, are volatile and are perceived by their aroma. Further on lipids are used as a carrier matrix for different compounds like vitamins and lipophilic aroma and are therefore responsible for the characteristic flavor. Lately some indications have been put forward, that lipids (unsaturated free fatty acids) might modulate other taste qualities or even give a taste per se (Gilbertson et al., 1997; Gilbertson, 1998). Already children learn to prefer tastes, flavors, and even textures that are associated with high energy density and therefore learn to select foods that are sweet, rich in lipids, or both (Drewnowski, 1997; Drewnowski and Greenwood, 1983; Mela, 1988; Drewnowski et al., 1989; Fisher and Birch, 1995; Gilbertson, 1998).

It is still not clear which stimuli and sensory systems contribute to the discrimination of lipid content in foods. Moreover the consumer-oriented approach was overlooked because there is very little information on underlying physicochemical processes occurring in-mouth that might govern fat perception. Therefore more and more research is focused on understanding multi-modal fat perception.

Fat texture perception

The perception of texture is a complex process involving the senses of vision, hearing, somesthesis and kinesthesis. Oral texture sensations of foods changes with time, from first bite to chewing and swallowing (Drewnowski, 1997). Understanding the relationship between food texture perception and food structure is of increasing importance for companies wishing to

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Table 1. Food properties and oral environment influencing factors to fat perception.

Sensory	Factors	Impact of food properties	Impact of oral environment
Bulk properties in liquid or semi-solid foods	Higher viscosity positively effects fat perception	Higher lipid content (Mela, 1988); Lipid type → higher saturation (Mela et al., 1994b); Decreased lipid particle; size/number ratio (Mela et al., 1994b); Type of emulsifier (Moore et al., 1998); Addition of tastants (sodium chloride, citric acid) (Barylko-Pikielna et al., 1994).	Pressure needed for spreading over oral surface (Stanley and Taylor, 1993) Saliva (sodium chloride, proteins,…) (van Vliet, 2002) Mouth temperature (van Vliet, 2002) Tongue movement creating different shear rates (Shama and Sherman, 1973)
Surface properties	Higher lubrication positively effects fat perception	Higher lipid content (Mela, 1988); Decreased fat droplets (Prinz et al., 2006a); Smaller particles (Prinz et al., 2006a); Specific thickeners (Prinz et al., 2006a); More viscous (de Wijk and Prinz, 2006).	Decrease in chewing cycles (Gavião et al., 2004) and chewing time (Brudevold et al., 1990); Starch breakdown (de Wijk and Prinz, 2006); Oral mucosal tissue and saliva (Ranc et al., 2006a).
Warmth or cooling	Slower heat transfer relates to higher fat content	Bite size (de Wijk et al., 2003a); Higher lipid content (Engelen et al., 2002); Lipid type (dairy and vegetable) (Hyvonen et al., 2003); Product temperature.	Oral temperature (Engelen et al., 2002).
Softness	Higher elastic properties	Higher lipid content (Adapa et al., 2000).	
Taste and bulk properties	Texture-taste interactions	Tastants (sodium chloride, citric acid) (Barylko-Pikielna et al., 1994); Mobility of the tastant in the matrix (Kokini, 1987).	Saliva (sodium chloride, proteins,…) (van Vliet, 2002).
Flavor, aroma	Texture - aroma interactions	Blocking the aroma perception; Aroma components (Yackinous and Guinard, 2000); Aroma partition coefficients, lipids as a carrier (de Roos, 1997); Addition of lipid-associated flavors (Tepper and Kuang, 1996; Yackinous and Guinard, 2000); Lipid content, type, droplet size (Charles et al., 2000; Jacobsen et al., 1999; Malone and Appelqvist, 2003; Miettinen et al., 2002; Ohmes et al., 1998; Welty et al., 2001).	Mastication time; Swallowing pattern; Saliva composition and volume (van Ruth et al., 2002); Oral temperature (Engelen et al., 2003b).

produce texturally attractive food products. Texture perception takes place during the dynamic process of food breakdown in the mouth and is affected by oral processes, such as motility, saliva and temperature. To take these factors into account, a multidisciplinary approach is needed for studying the relationship between food structure and texture perception, combining sensory research, physiological studies and research into food physicochemical characteristics (de Wijk et al., 2006b; Kokini and van Aken, 2006). Recent developments in these three areas have potential for a better understanding of texture perception and its relationship with food structure (Wilkinson et al., 2000).

The oral cavity is very important for food texture perception. There is a high density of nerve fibers and receptors located in different regions of the oral cavity, such as the lips, palate, tongue, teeth and mucosa (Ringel and Ewanowski, 1965). Thresholds for detection of light touch are lowest on the tip of the tongue and hard palate. Together these sensory systems are responsible for detecting sensations of touch-pressure, pain, temperature, and joint position. Most of the texture sensations are perceived when the food is manipulated, for example deformed or moved. The touch-pressure sensory qualities (somaesthetic) are detected by several classes of rapidly and slowly adapting neural elements that respond to small deformations of the skin (Trulsson and Johansson, 2002). In addition, kinaesthetic sensations provide information on movement and position of the mandible, which is important when particle size, i.e. the shape of food before and during mastication, is determined (Imai et al., 1999; Trulsson and Johansson, 2002). Joint receptors contribute to the estimation of such food texture attributes as hardness (Christensen and Casper, 1987; Guinard and Mazzucchelli, 1996).

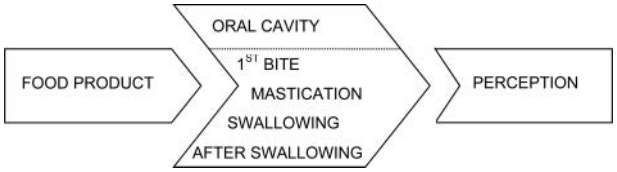

Figure 1. Importance of the dynamic process of perception during and after food manipulation in oral cavity.

Many studies have shown that texture is a parameter upon which lipid content is judged although it is not the only one (Drewnowski, 1997). Textures associated with changing lipid content can vary greatly, depending on the function of lipids in a particular food. For example, in liquid dairy products, where lipids are contained in emulsified micro globules, the perception of lipid content is guided by the sensory cues of smoothness and thickness. The cooling feel of lipids in the mouth is the characteristic feature of butter and cream. In contrast, the moistness of cakes and the juiciness of hamburgers are determined by the water-holding quality of lipids, and crispy and crunchy textures can be achieved by cooking in lipids at temperatures above that of boiling water. Sensory terms associated with fat texture in food are very product specific and are well discussed in published reviews (de Wijk et al., 2006b) and previous works (Cooper, 1987; Drewnowski, 1987; Weenen et al., 2003).

A more recent study summarized the results for texture mouthfeel and texture after feel attributes in the principal component analysis graph (de Wijk et al., 2006b). Texture attributes for vanilla custard desserts that varied in lipid content, starch content, and starch type, were summarized by three main sensory dimensions. The first dimension ran from roughness to creaminess sensations and was primarily driven by lipid content. The second dimension ran from melting to thickness and was primarily driven by starch content, whereas the third dimension running from airiness to heterogeneity was primarily driven by starch type. The first two dimensions corresponded well to results from a previous study (de Wijk et al., 2003b) in which sensory attributes were investigated for custard desserts that differed in lipid content, carrageenan content, and

starch content. The third dimension found in the present study is probably directly related to the starch types used, which elicit grainy and heterogeneous texture sensations. The results from instrumental measurements, together with the effects of ingredients, indicated that the first dimension, running from roughness to creaminess, was related to lubrication (Frøst and Janhøj, 2007). The second dimension, running from melting to thickness, was related to stimulus viscosity. Finally, the third dimension, running from airy to heterogeneous, was related to starch type (de Wijk et al., 2006a).

Terminology and sensory attributes to describe the textural contributions of lipids in food products are numerous and product specific. However, these attributes are related to what occurs in the mouth, whether this is on the mouth surface or in the bulk of the bolus.

Mechanisms for fat texture perception

Fat texture perception depends on various factors (Fig. 2). One of the most important is attributed to food structure. Due to the complexity, whenever lipid content and quality is changed, consequently the factors of fat characteristics are influenced (Kilcast and Clegg, 2002). Oral cavity characteristics and oral process, during which physical and chemical degradation takes place, plays an important role in fat perception (van Aken et al., 2007; de Wijk et al., 2011; Engelen and de Wijk, 2012). From current publications we cannot clearly allocate the influence of each factor, neither the rate of influence gained by interactions. In current sensory analysis it is common to use threshold approach for particular food characterization. This approach could be foreseen also in fat texture perception particularly, if we apply also physicochemical measurements to characterize factors impact. Consequently the factors will be transformed to parameters.

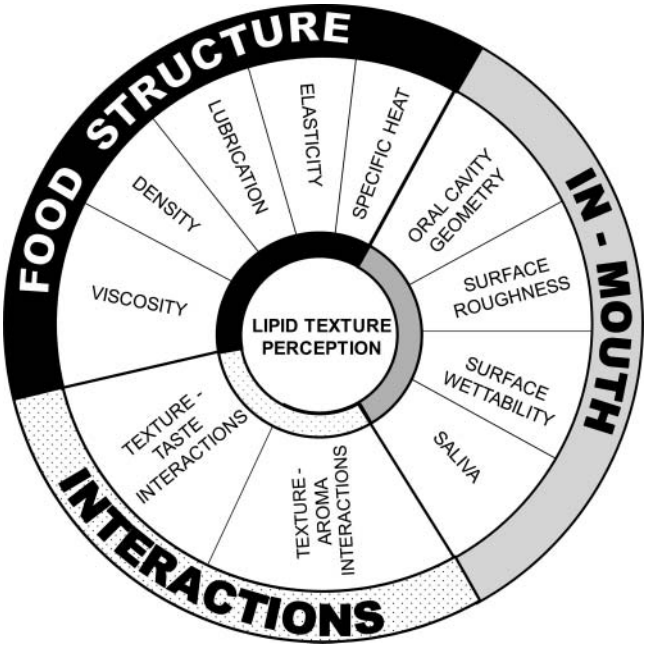

Figure 2. Factors of key importance for the lipid texture perception.

Viscosity as a parameter in the mechanism for texture perception

One of the textural attributes is described as thickness, which is linked to the viscosity of the product. Lipid content influences viscosity of food: higher lipid content (0–48%) results in viscosity increase and consequently fat content perception (Mela et al., 1994b). Fat content perception in liquid dairy products is largely attributable to textural properties, such as viscosity, sensed within the oral cavity. In dairy-based fluids creaminess and fat content ratings are closely positively correlated and driven by the fat content (Mela, 1988).

Lipid quality also influences viscosity, but does not necessarily improve the perception. In an experiment (Mela et al., 1994b), oil-in-water (o/w) emulsions were prepared with two oils differing in lipid saturation, sunflower oil and a highly saturated commercial cocoa butter substitute (HY5) and with different lipid contents (0–48%). The emulsions of sunflower oil and HY5 were compared when produced with homogenization pressure of 300 bar, where particle numbers and size distributions for the two oils were very similar, but viscosities substantially differ. A significant effect of lipid saturation on perceived fat content was shown; at the same lipid concentration (24–48%) the more viscous emulsions produced using a more saturated lipid source were perceived as higher in fat content. However, comparison of the stimuli at the same lipid content (0–24%) indicates that even though sunflower oil emulsions had 2–5 times lower viscosity than HY5 emulsions, they were perceived similar in fat content. Comparison of the stimuli at similar viscosity indicated that sunflower oil emulsions were perceived as substantially higher in fat content than HY5 emulsions, which might be due to the actual higher fat content.

Droplet size and size distribution in a food emulsion influences viscosity (Mela et al., 1994b). Oil-in-water (o/w) emulsions (0–50%) were prepared with sunflower oil and homogenized at pressures of 100 and 300 bar, which gave mean droplet size of between 0.3 and 2μm. Higher pressures, associated with a decreased lipid particle size/number ratio and increased viscosity, generated a slightly enhanced perception of lipid content.

Type of emulsifier or surfactant in a food emulsion influences viscosity. Another study has investigated the fundamental properties of emulsifiers that may contribute to the lipid-associated sensory attributes of emulsions (Moore et al., 1998). Model o/w emulsions were prepared with (0–48%) oil and emulsified with seven different emulsifiers; two proteins; sodium caseinate and whey protein, and five different sucrose esters. Emulsions were rated for perceived "fat content," "creaminess" and "thickness." Particle size, viscosity, thin film drainage, surface dilational modulus and interfacial tension were measured. The sensory results indicate significant main and interactive effects of lipid level and emulsifier type. At higher lipid levels, emulsions prepared with sodium caseinate and whey protein emulsifiers had higher viscosities and higher sensory scores than those prepared with the sucrose esters. Results indicate that emulsifier type has a significant effect on the sensory properties of o/w emulsions, and relationships between instrumental and sensory measurements suggest that this may be due to the interfacial properties of emulsifiers at the oil/water interface.

Taste compounds influence viscosity. Fifty percent o/w and water-in-oil (w/o) emulsions were prepared with sunflower oil and sucrose stearate as emulsifier. Addition of sodium chloride and citric acid increased perceived viscosity of both emulsion types and this effect was most pronounced in o/w emulsions. Perceived viscosity for sodium chloride was positively correlated with measured viscosity (25°C, 40 s^{-1}) in contrast to citric acid which showed a negative correlation between perceived and measured viscosity. Increase of measured viscosity (0.5–2.0 Pas) was due to flocculation in both types of emulsion after addition of electrolytes (sodium chloride and citric acid). The addition of sucrose to both emulsions did not change measured or perceived viscosity (Barylko-Pikielna et al., 1994).

Interaction of viscosity with the oral cavity

Oral viscosity plays an important role in the textural appreciation of fluid and semisolid foods (van Aken et al., 2011). Viscosity at an oscillatory frequency of 50 rad*s^{-1} had the highest correlation with assessed thickness of texture, but there is still much speculation as to which forces actually operate in the mouth (Stanley and Taylor, 1993). Shama and Sherman concluded that: a wide range of shear rates are involved in the mouth (from 10 to 1000 s^{-1}); the sensory evaluation of viscosity does not occur at a constant shear rate; effective shear rate strongly depends on the viscosity of the product (Shama and Sherman, 1973). The critical point of viscosity perception might be upon the introduction of liquid in the mouth. While fluids with low viscosity will spread faster and over longer distances, fluids with higher viscosity will spread slower and over shorter distances. So, pressure required to initiate significant flow might be another criterion for viscosity evaluation. Evaluation of oral viscosity depends on parameters such as shear rate, shear stress, temperature and flow properties of the food product. Additionally, as stressed by various authors, factors in the mouth, including saliva, may have a significant influence on the perceived rheology of fluid and semisolids (Stanley and Taylor, 1993; van Vliet, 2002).

The development of physical predictions for viscosity assessments first requires approximating the physical structure of the mouth, and then solving the appropriate physical equations for this geometry. It was assumed that liquid perception in the mouth takes place largely between the tongue and the palate (Kokini et al., 1977).

Perhaps sodium chloride in the saliva could also influence the viscosity of emulsion in the mouth. Several in-mouth parameters like temperature, shear rate, yield stress, oral surfaces, saliva flow and composition might significantly change viscosity of a liquid introduced in the mouth. Therefore viscosity, which occurs in the mouth, might not be correlated to the measured viscosity, which often excludes some of these parameters.

Lubrication as a parameter in the mechanism for texture perception

Lubrication is the ability of a substance placed between two surfaces to reduce friction that is the resistive force that occurs when two surfaces travel along each other when forced together. Water in the saliva moistens the food particles, whereas the salivary mucins as lubricants bind masticated food into a coherent and slippery bolus that can be easily swallowed (Pedersen et al., 2002). Lipids are widely used as lubricants and therefore may ease bolus formation, when present in food. Increased lipid content resulted in lower sensations of roughness, higher sensations of creaminess, and lower friction, suggesting that lubrication is the mechanism by which lipids affect oral texture in low-fat foods (Prinz and Lucas, 2000). Gavião et al. (2004) indicated that addition of butter may decrease needed chewing cycles by facilitating bolus formation and lubrication of the food. The same tendency was shown for chewing time, which decreased with increased lipid content (Brudevold et al., 1990). Instrumentally measured in vitro friction of semisolid foods (starch-based custard desserts) was correlated to oral texture sensations (de Wijk and Prinz, 2005). A food's lubrication properties are affected by its fat content, fat droplet size, particle size and shape and thickener. Foods with lower fat contents, larger fat droplets, larger particles and specific thickeners exhibited higher friction while their creaminess and fattiness sensations, associated with good lubrication, are reduced (Prinz et al., 2006a). Smaller droplets may be less deformable than larger ones, which results in a smaller contact area and consequently in reduced friction (Prinz et al., 2006a). Lipids are especially effective for the reduction of friction at relatively low levels (between 0% and 5%), but this effect continues to grow up to lipid contents as high as 10% or even 20%. At even higher lipid contents, as typically found in high fat products such as dressings and mayonnaises, friction seems no longer related to perceived roughness but may still relate to certain lipid-based attributes such as fatty after-feel. Friction is not only inversely related to lipid content but also to the viscosity of foodstuffs, i.e., foods with relatively high viscosities (above about 100 mPa*s) result in less friction than do those with lower viscosities. Starch breakdown by salivary amylase in low-fat foods resulted in reduced friction, possibly through the release of lipids from the starch food matrix, and the migration of lipids to the surface of the bolus where it becomes available for lubrication. The release of oil droplets from the matrix due to breakdown of starch is offering open field for research because only few articles are covering effect of degradation on ease of release of oil droplets from the matrix (Engelen et al., 2003a; de Wijk et al., 2006a; Janssen et al., 2009). Roughness and dryness sensations, associated with poor lubrication, are increased (de Wijk and Prinz, 2006).

Interaction of lubrication with oral cavity

Several studies investigated the influence of oral environment on friction coefficient measurements (Ranc et al., 2006a; Ranc et al., 2006b; Prinz et al., 2007a). Prinz and Lucas, (2000) demonstrated decrease in viscosity of saliva by addition of tannic acid, which resulted in an increased friction in vitro. They focused on the reduction of salivary viscosity while ignoring the role of the precipitate itself. However, the reverse position may also be valid, i.e., that it is the presence of the particulate precipitate that causes the sensation of roughness rather the reduction of salivary viscosity. No evidence was found for reduced lubrication as a result of reduced salivary viscosity. Also salivary mucins do not seem to influence lubrication. Astringency may be related to lower lubrication and increased friction caused by particles, either resulting from precipitation

of salivary PRPs or from flocculation of dead cells. Salivary friction was unrelated to salivary viscosity (de Wijk and Prinz, 2005). One of the mechanisms affecting lipid-related texture attributes is lubrication by lipids, more precisely by the number and the size of the droplets. Friction increased with increasing lipid droplet size and decreasing lipid content. Oral mucosal tissue and saliva strongly influence this parameter.

A study showed the effect of surface structure on frictional behavior of fluids (Ranc et al., 2006b). Friction is affected by a complex combination of factors including surface properties such as wettability and roughness, as well the contact pressure and the build up of a lubricating film. Depending on the surface structure the behavior of fluids is different. Therefore it is important to characterise the oral structure in order to predict lubrication. This was done in a following study (Ranc et al., 2006a), where the tongue surface showed a hydrophobic tendency and was weakly polar. The salivary layer made the surface of the tongue significantly more hydrophilic. Moreover, the presence of a salivary layer reduced the dynamic coefficient of friction by a factor of 1.6, indicating the lubricant function of saliva.

Lipids are well known lubricants in different systems. Their lubricating properties are correlated to viscosity, droplet size and lipid content. Therefore lubrication is one of the parameters upon which lipids are perceived. Friction and wetting properties of the tongue surface provide valuable insight into how the tongue interacts with other surfaces as a function of the coating. Saliva is an important lubricant of the oral cavity and influences friction depending on the food components.

Specific heat as a parameter in the mechanism for texture perception

It is thought that another mechanism to detect lipids could be the temperature change of the liquid in the mouth (Prinz et al., 2007b). Smaller bite sizes of cold custard dessert were judged warmer than larger bite sizes (2–11mL) (de Wijk et al., 2003a). This may come from the difference in the rate of heat transfer between the volumes. Smaller volumes are heated up to the oral cavity temperature more quickly than bigger volumes and therefore one perceived as much warmer. A further study indicated a correlation between perceived temperature and lipid content (Engelen et al., 2002), where a product with higher lipid content was perceived as warmer. The thermo receptors in the mouth are very sensitive to changes in temperature (Kadohisa et al., 2004; Stevens and Choo, 1998) and water and oil have different thermal properties, which might lead to different temperature sensations of a foodstuff during oral processing.

Physical melting may contribute to the greater sensation of lipid content. Emulsions prepared with a commercial cocoa fat (0–48% o/w) were evaluated at combinations of sample and mouth temperature of 20 and 36°C. Oral temperatures were manipulated by repeated cold and warm water rinses prior to assessments. There were no significant differences among these treatments on perceived fat content of the samples. No difference in judging fat content was observed between colder, more viscous samples and warmer, less viscous samples (Mela et al., 1994a). However, differences in viscosity of this magnitude have been shown to affect differences in fat content (Mela et al., 1994b). Another study examined the effect of oral (10, 22 or 35°C) and product (27, 35 and 43°C) temperature on the fat of texture and flavor attributes (Engelen et al., 2003b). The products, high and low-fat custard dessert and mayonnaise, were evaluated at different product temperatures in combination with different oral temperatures. Results showed that modulation of product and oral temperature had significant effects on a number of attributes. 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. In conclusion, the effect of oral temperature on the perception of sensory attributes in semisolids was small, but present, while product temperature influenced the ratings greatly.

When the product is solid at lower temperatures, the lipids might be perceived as different in melting patterns. Another study indicated that lipid level (0–18%) has more effect on the perceived melting of ice cream than lipid type (dairy and vegetable) (Hyvonen et al., 2003). The melting behavior of partly hydrogenated vegetable lipids and dairy lipids was probably not so different that it could be clearly perceived in ice-cream. Whereas a slight effect of difference in lipid type (dairy and vegetable) on melting pattern was indicated in table spreads (Tuorila and Vainio, 1993).The higher lipid content seems to retard melting (Hyvonen et al., 2003). Roland et al. 1999 reported discrepancy between physical and sensory measurements of melting characteristics of ice creams (Roland et al., 1999). Increasing lipid content increased physical melting rate, when sensory melting of the ice cream was decreased. The effect of total solids via melting point most likely is essential for the rate of melting as physically determined. Melting in the mouth includes the sensation of liquefying of both ice and lipid crystals. Consequently ice cream with higher lipid content is perceived to melt slower. Ice crystals melt at lower temperatures than lipid crystals.

Lipid heat transfer is different from water. Therefore it seems important to mimic in reduced-fat foods heat transfer of full-fat foods. Knowledge on surface area of oral cavity and its capacity to transfer heat is beneficial in understanding to what extent this effect influences perception.

Elasticity as a parameter in the mechanism for texture perception

Ice cream mixes and frozen ice creams at different milk lipid levels (6–12%) plus a protein-based fat replacer, and a carbohydrate-based fat replacer were evaluated for viscoelastic properties (Adapa et al., 2000). Elastic properties of the ice cream mixes decreased as lipid content decreased. Even fat replacers did not enhance the elastic properties of the ice cream mixes. The amount of lipids and the degree of lipid destabilization affected the elasticity in the frozen product. Even though the ice creams did not have significant elastic properties, when compared as a group the samples with higher lipid content had higher elastic properties. The addition of protein-based and carbohydrate-based fat replacers did not enhance the elastic properties of the ice creams but did increase the viscous properties.

Texture-taste interactions as a mechanism for texture perception

In a normal eating situation, interactions between texture and taste take place. One of the most well-known texture-taste interactions is that increasing viscosity reduces perceived taste intensity (Pangborn et al., 1978). For example, in the case of sweetness, produced by sucrose and fructose, taste reduction caused by increasing viscosity is based on the physiologic fact that to be tasted the sugar compound must diffuse to the surface of the taste buds on the tongue. The diffusion rate is dependent on the mobility of the tastant in the matrix and thus depends on the concentration of the tastant and the rheological properties of the thickener used (Kokini, 1987). Furthermore, different tastants have been reported to have effects on perceived textures. Sodium chloride and citric acid changed perceived viscosity of emulsions (50% o/w and w/o) and this effect was most pronounced in o/w emulsions (Barylko-Pikielna et al., 1994).

Texture-aroma interactions as a mechanism for texture perception

Aroma is obtained retro-nasally in the mouth during eating. Lipids can modify the perception of a food's flavor (taste, smell, and trigeminal components) (Mela, 1988). Blocking the aroma perception with a nose clip significantly reduces the fattiness ratings for all products, indicating that the aroma components of lipid-based foods influence fat perception (Yackinous and Guinard, 2000). There might be a chemosensory or tactile mechanism in the oronasal region of humans for detecting some aspect of the chemical composition of dietary lipids, or a component derived from or carried in lipids, that elicits fat perception (Mattes, 1996). Most aroma compounds in food are lipid soluble, and partition coefficients dictate that they will be associated with the lipid phase at equilibrium; thus, lipids act as a carrier for these flavors (de Roos, 1997). The matrix of the food (dependent on the amount and type of fat in the system) greatly affects flavor release rates and patterns, as well as aroma quality and aroma intensity of reduced-fat products (Ohmes et al., 1998; Welty et al., 2001; Malone and Appelqvist, 2003). Adding lipid-associated flavors (butter, cream, and grilled meat) to foods might enhance fat perception. For example the addition of a cream flavor to a model milk system enhanced the perception of fattiness (Tepper and Kuang, 1996). Another study showed a successful enhancement of the impression of fattiness for mashed potatoes and potato chips by adding higher levels of fatty-type flavors to these systems (Yackinous and Guinard, 2000). Because this enhancing effect is product-specific, extensive bench work and appropriateness studies with consumers should be performed in order to find appropriate flavor for certain product.

Influence of lipid content and composition on the aroma release

In general, an increase in food viscosity reduces perceived flavor intensity (Hollowood et al., 2002). This may partly be due to the total surface area of a firm sample available for aroma release increasing at a slower rate during mastication than that of a fragile sample. Thus, the total mastication time of firm samples is also longer than that needed for fragile samples (Wilson and Brown, 1997). The effect of lipids as a solvent of nonpolar aroma compounds was demonstrated, with increasing lipid content the release of linalool was decreased when measured using sensory evaluation and static headspace gas chromatography (Miettinen et al., 2002). The opposite was observed for the release of diacetyl, a more polar compound; it was slightly more retained in the aqueous than in the oil matrix. Lipids may not be as critical in the release of diacetyl as it is in the case of linalool. This is in accordance with earlier studies. Differences in timing of intensity perception of the retronasal aroma of a nonpolar (linalool) versus polar (diacetyl) compound when the matrix (milk) lipid content was varied (0–10% rapeseed oil) were studied. With increasing lipid content, linalool was considerably retained in the matrix, while the release of diacetyl was not affected. The observed temporal release of linalool partly challenges the often-repeated statement that increase of lipids results in a slower (Brauss et al,. 1999) and prolonged (Rosin and Tuorila, 1992) aroma release (Miettinen et al., 2003). Some studies have reported the opposite (Mialon and Ebeler, 1997; Guinard et al., 2002).

Lipid content slightly affected the perceived rate of flavor release and flavor intensity of strawberry-flavored ice cream (Hyvonen et al., 2003). Total absence of lipids caused significant changes in aroma and flavor profiles of the ice cream. In ice creams containing 9% or more lipids the strawberry aroma and flavor were perceived as typical and only slight differences were noticed between variants. In the same study, differences in flavor release depended on a lipid quality: a slightly faster flavor release was observed from ice cream made with more unsaturated vegetable lipids.

Influence of droplet size on aroma release

The influence of compositional and structural properties of o/w emulsions on aroma release ware examined under mouth conditions (van Ruth et al., 2002). Changes in particle diameter (0.6–1 μm) had a considerable effect an aroma release. Lipid fraction, emulsifier fraction, and particle diameter affected the kinetic components of aroma release, which could partially be attributed to changes in viscosity.

The dispersion of the droplet size (20–100 μm) in salad dressing models (50% oil-in-vinegar emulsions) had a small effect on flavor perception: when the droplet size was increased, lemon smell and citrus aroma significantly increased, whereas the egg note, mustard, and butter aroma significantly decreased (Charles et al., 2000). Reduced droplet size (0.7–6 μm), resulting from higher homogenization pressure, enhanced the release of linalool but had no effect on diacetyl (Miettinen et al., 2002).

Reduced droplet size results in an increased total surface area of the droplets, which may increase binding/entrapment of the volatiles at the interface assuming that the amount of emulsifier is sufficient to cover the smaller droplets formed (Jacobsen et al., 1999). The increased surface area available for volatilising may enhance the release of hydrophobic compounds (Charles et al., 2000). The effect of droplet size is likely to be very specific, depending on the nature of the aroma compound and the type and amount of the surface-active agent

used (Jacobsen et al., 1999; Charles et al., 2000; Miettinen et al., 2002).

Role of saliva in fat percpetion

Saliva is a complex fluid produced by a number of specialized glands which discharge into the oral cavity of humans. Most of the saliva is produced by the major salivary glands (parotid, submandibular, and sublingual), but a small contribution is made by the numerous small labial, buccal, palatal and lingual glands which line the mouth (Fig. 4). Many taste buds are localized in the trenches of the foliate and circumvallate papillae, where the lingual minor salivary glands (von Ebner's glands) secrete saliva (Fig. 3). Taste buds, situated at the surface of the anterior part of the tongue and soft palate, are bathed with the mixed saliva secreted mainly by the three major salivary glands (Matsuo, 2000). Taste perception occurs in specialized neuroepithelial receptor cells that are bundled in the taste buds in the lingual epithelium. Small tubulo-alveolar salivary glands, the von Ebner's glands, secrete into troughs, in direct contact with the taste buds. This saliva secretion is thought to be essential for the concentration and delivery of sapid molecules in the gustatory system and for clearing the tongue surface of taste substances, which would otherwise cause a long-lasting taste sensation (Schmale et al., 1990).

Effect of saliva during food manipulation in oral cavity:

- Protection to the oral and perioral tissues
- Bolus formation
- Lubrication with mucins and glycoproteins to facilitate swallowing
- The clearing of residues
- Buffering acid intake with bicarbonate
- Digestion, initiation with enzymes
- Solvent and transporter for taste substances

Saliva composition

Human salivary glands secrete 1000–1500mL per day of saliva. Mixed saliva consists mainly of the secretions of submandibular (65%), parotid (23%), and sublingual (4%) glands, the remaining 8% being provided by the minor numerous glands. These proportions are a function of the type, intensity and duration of stimulation. Normally during eating the parotid glands are stimulated and they secrete the major portion of whole saliva. Saliva is a dilute aqueous fluid containing both electrolytes and protein with an osmolality less than or equal to that of plasma (Table 2). In general, saliva contains the usual electrolytes of the body fluids, the principal ions being sodium, potassium, calcium, chloride and bicarbonate. Sodium, potassium, and chloride are the most important ions for maintaining the ionic strength of saliva. Saliva has a buffer capacity which neutralizes acids in the mouth. This capacity is based on several systems such as the phosphate system and the carbonic acid/bicarbonate system. In unstimulated saliva, the concentration of inorganic phosphate is rather high while the concentration of carbonic acid/bicarbonate system is low. The carbonic acid/bicarbonate system is the most important buffer in stimulated saliva due to its higher concentration.

Proteins in saliva

The proteins in saliva comprise approximately of 300 mg/100 mL, which is about 3% of the protein concentration in plasma. They include enzymes, immunoglobulins, glycoproteins and certain polypeptides and oligopeptides (Edgar, 1992). Saliva from different glands differs in composition of proteins (Fig. 4), which vary tremendously in their size (Fig. 5) Several proteins were identified representing various groups (Ghafouri et al., 2003; Amado et al., 2005; Hardt et al., 2005; Hirtz et al., 2005; Hu et al., 2005; Hu et al., 2007; Huq et al., 2007; Oppenheim et al., 2007).

In a later study from Vitorino et al. they could not identify fatty acid-binding protein, perhaps due to suppression by cystatins of tryptic peptidase signals, which are in the same gel region (Vitorino et al., 2004).

Saliva contains many different proteins, only a few of them are known (Table 3). Many of them are present in minor quantities and even their role is yet unknown, they may be important during oral food processing. Proteins are amphiphilic molecules which have a tendency to go to the interface of two immiscible fluids. Therefore salivary proteins might ease distribution of lipids in saliva.

Alpha-amylase, the major digestive enzyme of saliva, is present in parotid and submandibular saliva. It is a glycoprotein. Six isoenzymes exist; the distribution of isoenzymes from saliva differs from that of pancreatic juice. The enzyme hydrolyses the α-1:4 glycosidic bond between glucose units in the polysaccharide chain of starch; hydrolysis takes place anywhere along the chain (except where branching occurs), but is very slow for terminal glucose units. Thus the end-products of amylase digestion are mainly maltose together with oligosaccharides as well as some free glucose (Edgar, 1992).

The mucins are high molecular mass glycoproteins, in which proline and serine/threonine constitute up to 20–55% of total amino acids and are concentrated in one or several regions of the polypeptide. These serine/threonine residues are heavily glycosylated, and 40–80% of the mass of such mucins consists of O-linked oligosaccharides. The cysteines at the N- and C-ends may link mucin monomers by disulfide bridges forming linear mucin oligomers. Two types of mucins are present in human saliva: oligomeric mucin glycoprotein (MG1)—high molecular mass protein, and monomeric mucin glycoprotein (MG2)—low molecular mass protein. The submandibulary glands containing mucous cells (producing MG1) and serous cells (producing MG2) secrete 30% of the salivary mucins, while sublingual, labial and palatal glands (which contain mainly mucous cells) secrete 70%. Concentration of mucin secreted by sublingual glands is higher than that secreted by submandibulary glands, while the secretion of parotid glands is devoid of mucins (Zalewska et al., 2000). Up to 26% of the salivary proteins are mucins. The mucins of human saliva are extremely effective lubricants, which provide an effective barrier against desiccation and environmental insult. They control permeability of the mucosal surface, limit penetration of potential irritants and toxins to mucous cells, protect mucosal cell membranes against the proteases generated by bacteria in the bacterial plaques around the teeth and regulate colonization of the oral cavity by bacteria and viruses. Several studies have demonstrated that salivary glycoproteins are capable of in vitro

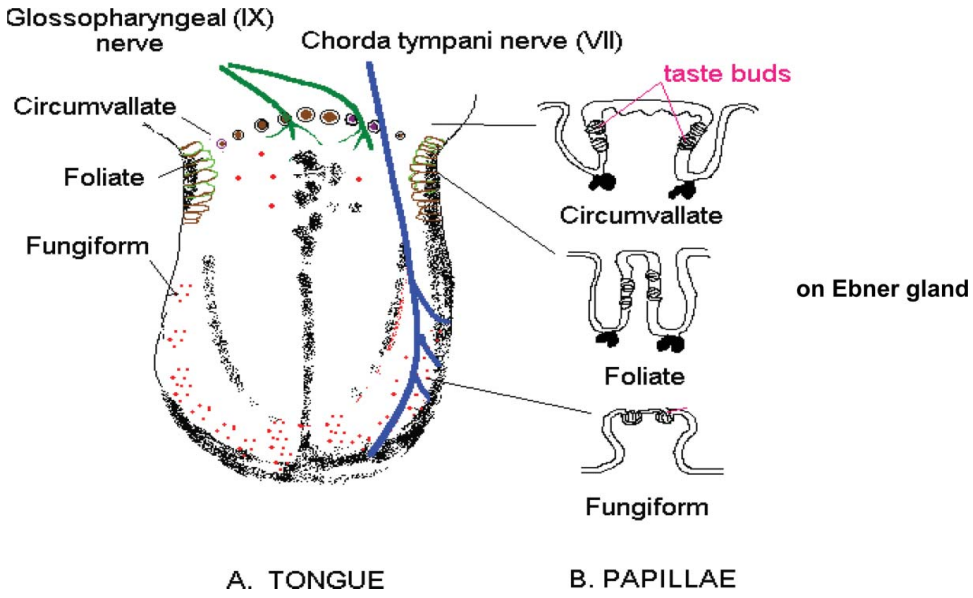

Figure 3. Different papillae with minor salivary gland on human tongue (Matsuo, 2000).

lubrication. On a molar basis, the relative lubricating ability of the various salivary glycoproteins tested was found to be MG1 > MG2 > the proline-rich glycoprotein present in the human parotid saliva. Both the viscosity and wetting properties of salivary substitutes augmented with animal mucins more closely approximated for values obtained for the human salivary secretions.

It was also suggested that acid stimulus might increased the mucins content of whole saliva, through the observation that acid stimulation influences the viscosity and elasticity of saliva (Stokes and Davies, 2007).

Shi et al. used bovine submaxillary gland mucin as an emulsifier to stabilize o/w emulsion systems. The surfactant property of mucin was investigated by surface tension measurements, which showed the bovine submaxillary gland mucin greatly reduces the surface tension of phosphate buffered saline. Compared to several synthetic surfactants, mucin showed comparable or better surface activity. Thus, mucin showed its ability to

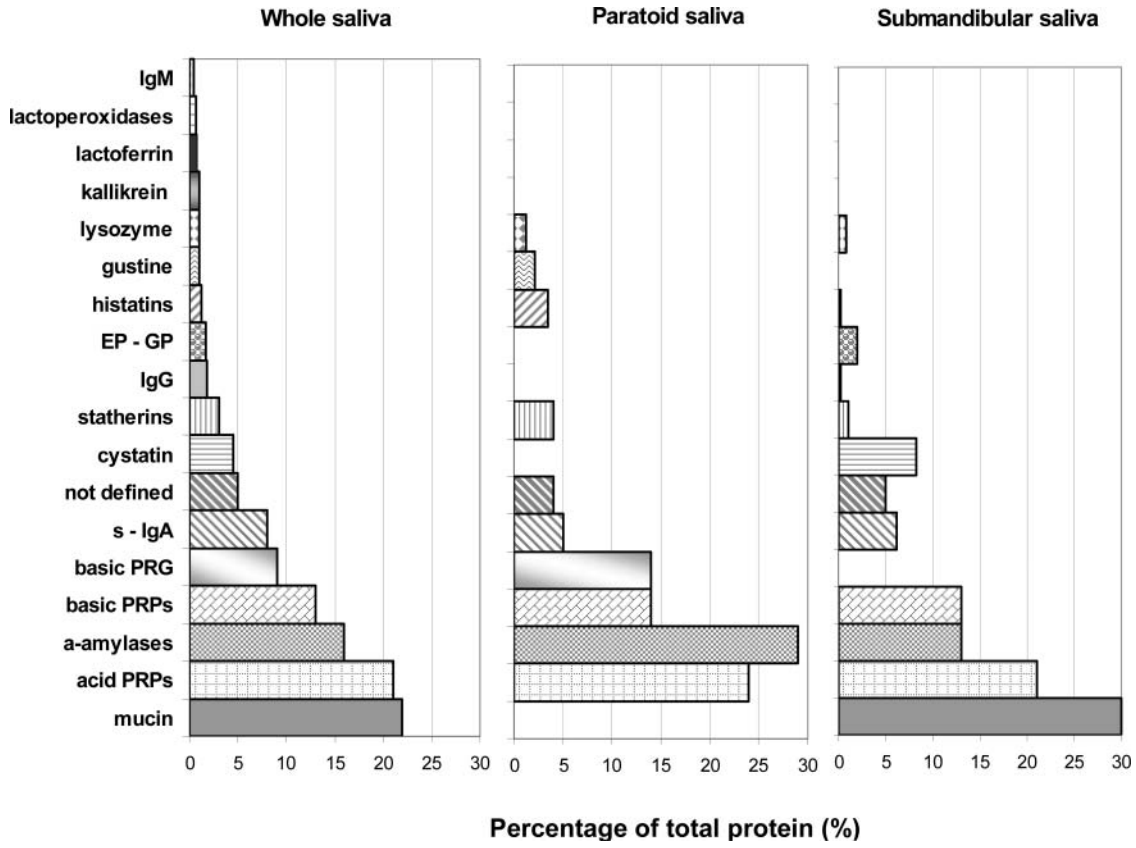

Figure 4. Percentage of total protein content from parotid, submandibular and whole saliva (Hőld et al., 1995; Larsson et al., 1996; Actis et al., 2005).

Table 2. Saliva composition (Actis et al., 2005; Edgar, 1992; Hőld et al., 1995; Larsson et al., 1996).

Parameter	Unit	Whole Saliva
Volume	mL/day	500–1500
Rate of flow	mL/min	0.6 (0.1–1.8)
pH	/	6.7 (5.6–7.9)
Water	%	98 (97–99.5)
Dry Substance	mg/100 mL	600 (380)
Total protein		300 (150–640)
Mucin		270 (80–600)
Amino acids		0.1–40
Lipids		1.31 (0.33–15.6)
Cholesterol		7.5 (3–15)
Potassium	mMol/L	8–40
Sodium		5–100
Calcium		1.5–2
Phosphate		5.5–14
Chloride		5–70
Bicarbonate		0–6

establish more stable and more efficient o/w emulsion systems (Shi et al., 1999).

This is a good example of mucin being active at the interface, being able to form stable emulsion. Remaining question is: Does it also help in the mouth to disperse lipids to form a fatty mouth coating?

Mucins have been shown to induce flocculation of emulsions, which could suggest the role in the perception of texture attributes of foods (Vingerhoeds et al., 2005).

Proline-rich proteins (PRPs) are major components of parotid and submandibular saliva in humans as well as other animals. They can be divided into acidic, basic and glycosylated proteins. The primary structure of the acidic PRPs is unique and shows that the proteins do not belong to any known family of proteins. The PRPs are apparently synthesized by the acinar cells of the salivary glands and their phenotypic expression is under complex genetic control. The acidic PRPs strongly bind calcium which indicates that they may be important in maintaining the concentration of ionic calcium in saliva. Little is known about the function of glycosylated and basic proline-rich proteins.

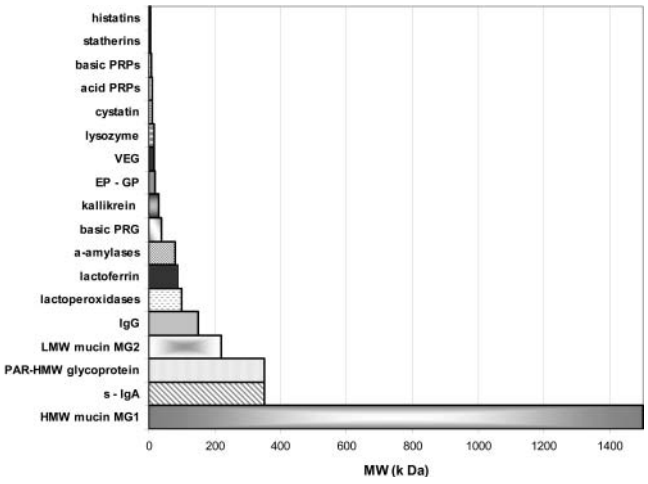

Figure 5. Large variation of protein size in saliva (Hőld et al., 1995; Larsson et al., 1996; Actis et al., 2005).

Table 3. Selection of proteins found in human saliva.

Abundant proteins expressed in different forms	alpha-amylase, immunoglobulin A, prolactin-inducible protein, zinc-alpha(2)-glycoprotein, cystatins (S, SA, D and SN);
Other proteins	interleukin-1 receptor antagonist, von Ebner's gland protein (lipocalin-1), calgranulin A and B (S100A and A9), beta(2)-microglobulin, glutathione S-transferase P,
Proteins identified for the first time in human saliva	fatty acid-binding protein, apolipoprotein A-1 certain isoforms of alpha-amylase prolactin-inducible protein

Tannins are polyphenolic compounds, widely distributed in plant-based foods. Salivary PRPs may act as a defence against the tannins by forming complexes with them and thereby preventing their interaction with other biological compounds and absorption from the intestinal canal. Basic PRPs effectively form insoluble complexes with condensed tannin and tannic acid, as opposed to acidic and glycosylated PRPs, (Lu and Bennick, 1998). Although described as tasteless, tannins can be detected orally by their astringency. However, the mechanism of oral detection and the effect of tannins on mastication and swallowing have been little investigated. Some in vitro tests showed that tannic acid significantly reduces the lubricating qualities of human saliva both by decreasing its viscosity and increasing friction, both factors lending support to the notion that astringency is a tactile phenomenon (Prinz and Lucas, 2000).

Lingual lipase is thought to be an auxiliary enzyme for lipid digestion and absorption in mammals; however, the reason for its secretion in the oral cavity is not known. Several authors indicated that during lipid consumption we might detect polyunsaturated free fatty acids, which give a lipid taste per se or perhaps by modulation of other tastes (Mattes, 1996; Gilbertson et al., 1997; Gilbertson, 1998; Mattes, 2001; Nasser et al., 2001; Kamphuis et al., 2003;). Lingual lipase might cleave these polyunsaturated free fatty acids from triglycerides in-mouth. A study on rats focused on the gustation and investigated the significance of lingual lipase in the perception of lipid taste by using orlistat, a potent lipase inhibitor. Five-minute two-bottle preference tests demonstrated that the addition of orlistat diminished the preference for triacylglycerides but not for free fatty acids (Kawai and Fushiki, 2003). These findings suggest that lingual lipase is released to hydrolyze triacylglycerides into free fatty acids that are perceived as a taste quality or to estimate the nutritive value of food. However, rat lingual lipase has a much higher activity than human lingual lipase.

The lingual serous glands of von Ebner, which secrete lingual lipase, are located close to the foliate and circumvallate papillae. Saliva secreted by these glands provides the immediate environment of the taste buds, and it has been hypothesized that it modulates taste perception. The SDS gel electrophoretic profile of von Ebner saliva revealed two protein bands of molecular mass 18,000 identified on Western blots as von Ebner gland proteins (VEG). Although lingual lipase activity was detected at very low levels (2.5 $\pm$ 0.5 mg/mL) by enzyme assay, this protein was not detected on Western blots. This might be due to insufficient sensitivity of the immunotechniques or that antibodies of lingual lipases are dissimilar between rat and human (Spielman et al., 1993).

Human tongue preparations contain lipolytic activity that is present in homogenates of the glandular region (von Ebner) beneath the cirumvallate papillae, and in secretions collected from the trough of the papillae. The lipolytic enzyme hydrolyzes long chain triglycerides to partial glycerides (di- and monoglyceride), glycerol, and free fatty acids at pH optimum 5.4. These findings suggest that in man the lingual serous glands secrete a lipase that acts in the stomach where it initiates the digestion of dietary lipids (Hamosh and Burns, 1977).

Considering that the pH in the mouth is normally around 7, lipolytic activity might be lower in the mouth. However, this activity might be still sufficient due to lipase secretion nearby taste buds allowing to each fatty acid released from triglycerides to interact with taste cells receptors. During the ingestion of sour food, salivary pH might decrease and therefore enhance the lipase activity. Moreover incomplete hydrolysis forms diglycerides and monoglycerides that have a high potential to stay at the oil/water interface and may help to distribute lipids in the mouth.

Lipocalins are carrier proteins for hydrophobic molecules in many biological fluids. In the oral sphere (nasal mucus, saliva, tears) they have an environmental biosensor function and are involved in the detection of odours and pheromones (Malnic et al., 1999). Olfaction involves the binding of small, hydrophobic, volatile molecules to receptors of the nasal neuroepithelia. It generates a cascade of neurological events that transmit the information to the olfactory bulbs projecting into the brain. The very first step in this process is the solubilization of these hydrophobic molecules in the hydrophilic nasal mucus. Odorant-binding proteins are thought to transport these molecules within the mucus (Pelosi, 1996). For the first time an identification of human lipocalins involved in odorant binding was reported (Lacazette et al., 2000). Salivary lipocalins might also be responsible for lowering surface tension of saliva (Nagyova and Tiffany, 1999).

VEG is a salivary protein secreted by the von Ebner's glands located around the circumvallate and foliate papilla of the human tongue that belongs to the lipocalin superfamily (Blaker et al., 1993). Sensory transduction in taste and olfaction, the principal chemical senses, seems to be mediated by membrane-associated proteins on the apical surfaces of the respective receptor cells. This protein was cloned and characterized (Schmale et al., 1990). Because of the ability to bind hydrophobic ligands, such as free fatty acids (Abduragimov et al., 2000), it has been suggested that the primary function of lipocalins is to accommodate lipophilic molecules in a hydrophilic environment, allowing transport of these molecules through body fluids toward their physiological receptors. The hypothesis has been put forth that the salivary VEG protein is involved in perception of bitter taste by binding lipophilic bitter compounds and transporting them to the taste buds (Garibotti et al., 1995).

The human VEG was expressed in *Escherichia coli* and purified to homogeneity. Recombinant VEG was found to be stable under acidic conditions, in the presence of alcohol, and at high temperatures. The denaturation temperature was 79°C at pH 3.5, with a denaturation enthalpy (ΔHd) of 160,600 J/mol). Fluorescence analysis and measurement of the denaturation temperature by circular dichroism did not detect any interaction between VEG and extremely bitter (denatonium benzoate, caffeine) or sweet (aspartame) compounds (Creuzenet and Mangroo, 1998). These results, contradictory to the previous hypothesis, suggest that VEG may not function as a shuttle for transfer of sapid molecules to taste receptors. However, recombinant protein might not always represent the original one.

Fatty acid binding protein has also been identified in human saliva (Ghafouri et al., 2003). The putative membrane fatty acid transporter protein and its mRNA, originally expressed in adipose tissue, were found in the tongue of rats. Northern blot analysis showed a significant expression of fatty acid transporter mRNA in the epithelial layer of circumvallate papillae. Immunohistochemical staining revealed that immunoreactivity of the fatty acid transporter is specifically localized in the apical part of taste bud cells, possibly gustatory cells, in the circumvallate papillae (Fukuwatari et al., 1997).

Lysozyme is an enzyme that destroys bacterial cell walls by hydrolysing the polysaccharide component of the cell wall. Lysozyme can be found in saliva and it is responsible for its antibacterial function. Lysozyme is very basic and strongly interacts with neutral or acidic proteins. In a mixture with other proteins lysozyme contributes to better foaming and emulsifying properties (Yampolskaya and Platikanov, 2006).

Lipids in saliva

The concentrations of total lipids in parotid, submandibular and whole stimulated saliva were 0.2, 0.9 and 1.3 mg/100 mL, respectively. Cholesteryl esters, cholesterol, triglycerides, diglycerides, monoglycerides and free fatty acids accounted for 96–99% of total salivary lipids. Thus, polar lipids, such as phospholipids, contributed only a minor fraction, indicating that lipids are not primarily of membrane origin. The content of free fatty acids and partial glycerides was high (Larsson et al., 1996). Although there is a small amount of lipids in saliva, they are incorporated with proteins. Over 50% of the total lipids of submandibular saliva were found in the fraction which contained mainly the high-molecular-weight glycoproteins. This fraction also contained most of the glycolipids, free fatty acids, phospholipids, and cholesterol. In parotid saliva, the fraction containing the basic glycoprotein (the major glycoprotein fraction of parotid saliva) contained 35% of the total saliva lipids and was enriched in phospholipids and cholesterol esters (Slomiany et al., 1983). Salivary proteins help to distribute lipids present in saliva; however none of the studies showed their role in incorporating food lipids introduced in the mouth.

Interaction of saliva with food emulsion

Food is exposed to a range of processing steps during consumption. It is broken down by chewing, mixed with saliva, heated or cooled to body temperature, air is introduced, and it comes into contact with oral surfaces and is exposed to complicated saliva flow profiles. Upon consumption of emulsions, processes such as dilution and phase inversion can occur; in addition, the lipids may change from the solid to the liquid form if they pass through its melting point during the course of consumption. The underlying physicochemical processes might be important to link these structural changes under oral conditions to sensorial attributes.

Influence of saliva on perceived thickness in the mouth by changing viscosity

The flow behavior of some Newtonian (glucose syrup) and non-Newtonian fluids (milk, melted ice-cream) was studied in vitro over a wide range of shear rates, both before and after mixing with human saliva (Parkinson and Sherman, 1971). It was shown that saliva increases the viscosity of liquids with low viscosity. For liquids with high viscosity there was a slight decrease of viscosity. It could be that some compounds in saliva interact with compounds in low viscous fluids and thereby increase the viscosity. However, the effect is not as pronounced in higher viscosity fluids, where dilution with saliva might have a greater impact on viscosity reduction. Saliva affects perceived viscosity, which is an important parameter to perceive texture of liquid foods (Stanley and Taylor, 1993). The shear rate which operates in the mouth during assessment of fluid with low viscosity can be as high as 1000 s^{-1} (Wood, 1968). At shear rates of this order many fluids exhibit turbulent flow and this leads to an apparent increase in viscosity. Parkinson proposed that if a fluid in the mouth flows between the tongue and the roof of the palate at high shear rate, then the sensory evaluation of low viscosity fluids could well be based on shear stresses developed in turbulent flow. Addition of saliva decreases the tendency of low viscosity Newtonian fluids to exhibit turbulent flow (Parkinson and Sherman, 1971). This might possibly be due to the salivary glycoproteins, because the same effect has been shown in many types of fluid systems for long chain, high molecular weight molecules (Gadd, 1966). The saliva decreases the tendency of fluids to exhibit turbulent flow at higher shear rates, which results in a viscosity decrease. In the case of low viscosity Newtonian fluids the viscosity is still higher due to the unknown effect of saliva.

The effect of adding saliva or a saliva-related fluid (alpha-amylase solution and water) to custard prior to ingestion on the sensory ratings of odour, flavor and lip-, tooth-, mouth- and after-feel sensations was investigated. The results show that addition of a fluid (saliva, alpha-amylase solution and water) affected the mouthfeel attributes of perceived melting, thickness and creamy. Perceived melting was the only attribute on which the type of fluid had an effect: saliva elicited a stronger melting effect than the alpha-amylase solution and water. The volume of the added fluid affected a number of attributes like thick, creamy mouthfeel and fatty after-feel (Engelen et al., 2003a).

Amount of glycoproteins in saliva varies during the day and also shows large differences between people. Consequently, it would be of interest to see if all saliva exhibits this tendency of increasing and decreasing viscosity of low and high viscosity fluids and to what extent. It would also be interesting to study the effect of saliva on lipid-based fluids as well as to identify glycoproteins in saliva that have a major impact on food viscosity.

Compounds in saliva lowering surface tension

Saliva constantly flushes the oral cavity 1000–1500 mL/day (Hőld et al., 1995). Nevertheless, after swallowing some remains and covers the oral surfaces. Evaluation of the mechanisms of adhesion in the oral cavity requires, among other things, knowledge of the strength of the surface tension, which allows saliva to be retained in the mouth. The mean surface tension of saliva was found to be 53.1 mN/m with a range between 47.8 and 59.1 mN/m (Glantz, 1970). Dispersion forces were measured by contact angle on polytetrafluoroethylene plates with a mean value of 28.7mN/m. Other studies have reported similar surface tensions (Braddock et al., 1970; Kirkness et al., 2000). The surface tension of commercial mucins, lipocalins, lysozyme, lactoferrin and secretory IgA, was closest to that of tears (Nagyova and Tiffany, 1999). Since all of these proteins are present in saliva, they may contribute to its surface tension.

Surface tension of saliva is one of key parameters governing wettability and adhesion of saliva to oral surfaces. Therefore it is of great interest to study which compounds in saliva lower surface tension. Mucins, as shown in tears, may be most important in lowering surface tension. However, it might be of interest to check the contribution of proline-rich protein, as one of the major proteins in saliva. Consequently to study the effect of those compounds in a lipophilic phase, such as oil, might help to understand some mechanisms that occur during mouth coating.

Formation of fatty mouth coating

Fatty mouth coating is a residue of food emulsion compounds on oral surface, which contribute to several sensory attributes. De Hoog et al. 2006 reported on an experimental study on the interactions of emulsions with oral surfaces. The behavior of the emulsion droplets at artificial and tongue surfaces and in the presence of saliva was visualised by confocal scanning light microscopy in a microrheological set up. This enabled the study of aggregation, adsorption and coalescence processes directly at the surfaces under quiescent or flow conditions that are very similar to in vivo conditions. It was observed that triglyceride emulsions stabilised by beta-lactoglobulin were strongly aggregated in the presence of saliva and sometimes also adhered to the surface. Coalescence was observed if the two surfaces (either rubber and glass or tongue and glass) were moved with respect to each other at a rate similar to the velocity of natural tongue movements and at a very small distance between the surfaces.

Suggested consecutive steps in destabilization of food emulsion in the mouth, which leads to fatty mouth coating (Fig. 6):

1. interaction with saliva causing aggregation of emulsion
2. interaction with emulsifiers and mucus layer or oral surface causing adsorption on a surface
3. coalescence on the surface, due to high shear stress between tongue and palate
4. formation of fatty mouth coating

Due to the rapid aggregation of food emulsions induced by saliva, the stability and behavior of the emulsion may be influenced to such an extent that it affects the mouthfeel and other sensory aspects of the emulsion. Moreover, in relation to after-feel, small amounts of the emulsions remain present in the oral cavity after swallowing, thereby increasing the interaction time with saliva (Vingerhoeds et al., 2005; de Jongh et al., 2006; Saint-Eve et al., 2006).

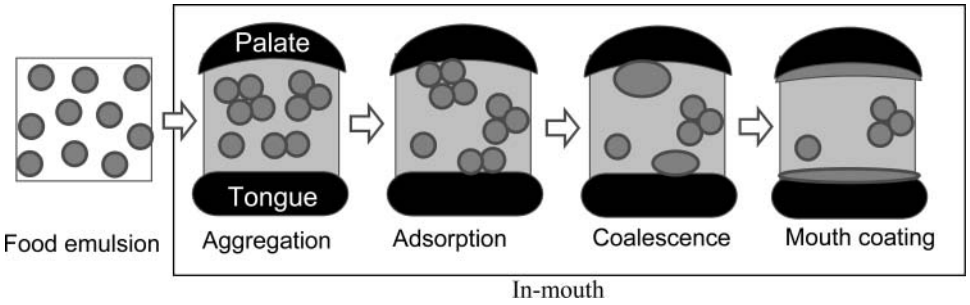

Figure 6. Suggested consecutive steps in destabilization of food emulsion in mouth, which leads to fatty mouth coating.

Aggregation of food emulsion in the presence of saliva

To study the interaction of saliva with emulsions in the oral cavity, emulsions were mixed in vitro with fresh, centrifuged saliva and examined by light microscopy (Vingerhoeds et al., 2005). Emulsion aggregation induced by saliva was observed. No significant difference was observed for saliva from different persons and the different types of emulsifiers (whey protein isolate, beta-Lactoglobulin, sodium caseinate, beta-casein and Tween 20). However, the overall appearance, i.e. the size of the droplet aggregates and speed of aggregation, which ranged from instantaneous up to 1 min, varied among the individual saliva samples. Aggregation usually occurred within seconds of mixing. This fast rate of aggregation suggests that that flocculation also occurs in vivo upon emulsion consumption (Vingerhoeds et al., 2005).

As mucin is one of the major protein fractions in saliva, it was hypothesized that this glycoprotein was causing the observed aggregation. The effect of mucin on emulsions was examined by addition of pig gastric mucin to model emulsions and again aggregation of the emulsions was clearly observed. In most cases, no aggregation was observed when emulsions were mixed with saliva from parotid glands, which does not contain mucins. The average mucin concentration in saliva is about 0.02 wt%, which is far below the critical aggregation concentration observed for pig gastric mucin (0.4 wt%). However, in vitro mixing of emulsions with whole saliva (in a ratio 1:1) also clearly resulted in emulsion aggregation. The lower critical flocculation concentration might partly be explained by the occurrence of large aggregates of salivary mucins alone or after interaction with other salivary proteins or even bacteria (Vingerhoeds et al., 2005).

The apparent viscosity of whey protein isolate emulsions, pig gastric mucin solutions and their mixtures were measured as a function of applied shear rate. Both the plain emulsion and the pig gastric mucin solution showed Newtonian behavior over the range of shear rates applied. Upon addition of pig gastric mucin, the whey protein isolate-emulsion showed an increase in viscosity at low shear rates, which can be ascribed to the presence of droplet aggregates, which occupy more volume than the non-aggregated emulsion droplets. At increasing shear rates these aggregates are broken up into smaller ones, leading to a decrease in effective particle volume and emulsion viscosity. Aggregation is reversible as upon lowering the shear rate, the aggregates are formed again and the viscosity increases (Vingerhoeds et al., 2005). The observed flocculation, which affects emulsion rheology, might also influence sensory properties of liquid emulsions.

At physiological pH, salivary mucins as well as other salivary proteins, are negatively charged. Aggregation might be due to an electrostatic interaction between these proteins and the positively charged emulsifiers on the droplet surface (Silletti et al., 2007a). The sign and charge density on the emulsion droplets determine the behavior of saliva/emulsion mixtures (Silletti et al., 2007a):

- Strongly negatively charged emulsions do not flocculate.
- Neutral and weakly negatively charged emulsions flocculate, mainly due to depletion interactions.
- Positively charged emulsions flocculate by electrostatic attraction.

Confocal scanning laser microscopy (CSLM) showed formation of complexes between salivary proteins and lysozyme adsorbed at the oil–water interface and lysozyme in solution as well (Silletti et al., 2007b). It was concluded that electrostatic attraction is the main driving force for complex formation between saliva components and lysozyme adsorbed at the oil droplets and in solution.

So far only mucins have been identified as inducing aggregation of food emulsions, however it seems that other salivary components also promote it. However, the authors did not check the influence of ion strength by addition of sodium chloride, which would probable influence emulsion flocculation. There is evidence of saliva inducing emulsion flocculation, but the effect on perception is not known.

Adsorption-effect of emulsifier binding to the oral epithelium

Soft solid foods such as mayonnaise or dressings that are high in oil content coat the oral mucosa to form a thin film (mouth coating) (Appelqvist et al., 2004). This can be achieved with standard emulsions at high lipid content or for lower lipid content emulsions by increasing the viscosity of the emulsion. An alternative approach is to design emulsions with a stabiliser that interacts with the oral mucosa in order to enhance surface deposition and increase the residence time of the oil (even at low lipid content). The interfacial interactions between various emulsions and a mucin covered solid substrate were investigated using evanescent wave spectroscopy. This technology allowed the observation of real-time, deposition kinetics for the processes of molecular physo- or chemo-sorption of emulsifiers and emulsions. To determine the extent to which different emulsifiers may influence the deposition of oil onto the oral

mucosa, a range of 1% o/w emulsions stabilised with chitosan, egg-yolk and Tween 60, were evaluated.

The results indicated that the chitosan emulsion adsorbs onto the mucin film surface and has a very strong affinity for the substrate. The mechanism by which the chitosan adsorbed to the mucin film was through electrostatic interaction. At pH 3.5, the pH of the binding experiment, the chitosan emulsifier was positively charged and was electrostatically attracted to the mucin which had a net negative charge. Moreover, the rate at which the oil droplets adsorbed onto the mucin film is governed by the strength of the electrostatic forces that can be controlled through modification of the ionic environment and the pH of the system. At higher pH the negative charge on the mucin film will be greater and the overall electrostatic force increases resulting in a faster adsorption rate. Alternatively high levels of salt present will screen the electrostatic interaction and reduce the binding.

The egg yolk emulsion adsorbed less than the chitosan emulsion onto the mucin film. It is likely that the proteins within the egg yolk were adsorbed onto the mucin surface because they were slightly positively charged.

On the other hand, the Tween 60 emulsion did not show any adsorption onto the mucin covered surface, and in fact, a slightly negative slope to the binding curve was observed. Perhaps this is due to the non-ionic nature of Tween 60, which led to no electrostatic interactions and, hydrogen bonding if any, did not give rise to any significant binding to the mucin substrate (Appelqvist et al., 2004).

To assess the impact of oil deposition and residence time on aftertaste delivery non-adsorbing emulsions (Tween 60) and emulsions with a high affinity for the oral mucosa (chitosan) were compared using atmospheric pressure chemical ionization–mass spectrometry-breath analysis. The release of flavor from the mouth-coating could be modified by designing emulsions that interact with the oral mucosa (Appelqvist et al., 2004). The data clearly showed that that rate of release observed with emulsions stabilised with Tween 60 was much higher than those of the chitosan stabilised emulsions, particularly at low oil contents. The benefit obtained with the chitosan was that it allowed more oil to be deposited onto the oral mucosa (hence the lower rate constant) and increased the residence time of the oil droplets in the mouth. As a consequence of more oil being deposited on the oral surface, there was more flavor available to be delivered as an aftertaste. This suggested that at higher lipid levels (>15%) the deposition of oil reaches a limiting value irrespective of the oil content or emulsifier, and implied that mechanisms other than electrostatic binding to the mucin occur at these higher lipid levels.

Another study determined whether milk proteins such as β-casein could adsorb onto a mucin film and related this to sensorial roughness (Malone et al., 2003). Skimmed milk was pasteurised and treated with ultra high temperature and then injected into the mucin covered cell to study kinetics of binding. The results showed that different heat treatments of the skimmed milk affected binding to the mucin film. The pasteurised milk binds very rapidly and strongly to the mucin film eventually plateauing. This was compared to the UHT milk, which showed no binding. The effect of higher temperature treatment on the milk was to reduce significantly its ability to adsorb to mucin. One explanation for the difference in binding is that in pasteurised milk the β−−casein that covers the casein micelles can interact strongly with the mucin glycoprotein and shows strong deposition onto the mucin film. When the milk is heat treated the whey protein that makes up the continuous phase can unfold and can migrate to the surface of the β-casein effectively enveloping the micelle and thereby acting as a screen and preventing binding. To test the effect of binding of protein with mucin on mouthfeel, the heat-treated milk systems were sensorial assessed by an untrained panel and differences in roughness was determined. The results show that the pasteurised milk was assessed as being rougher than the UHT treated milk and therefore the preliminary observation is that food products that adsorb to the mucosal mucin film are likely to lead to a rough mouthfeel.

Ex vivo (Confocal Raman Spectroscopy; Confocal Scanning Laser Microscopy) experiments using pig's tongue surfaces in combination with human in vivo experiments reveal that protein-poor (unstable) emulsions are retained more at the tongue than protein-rich (stable) emulsions (Dresselhuis et al. 2008). Furthermore, the layer formed by adhering protein-poor droplets is more stable against rinsing.

Adsorption of oil droplets onto the mucin film is governed by the strength of the electrostatic forces that can be controlled through modification of the ionic environment and the pH of the system. This might result in more oil binding to the mucus, which forms the mouth coating, moreover persisting as an aftertaste.

Coalescence

Coalescence was observed in emulsions stabilised by β-lactoglobulin and mixed with saliva, if the two surfaces representing tongue and palate (either rubber and glass or tongue and glass) were moved with respect to each other at a rate similar to the velocity of natural tongue movements and at a very small distance between the surfaces (de Hoog et al., 2006).

Little is known about the formation of mouth coating. There is no information on its structure, few assumptions are made on possible interactions and mechanisms, which might contribute to its formation.

Novel techniques to study the residues on oral surfaces

To understand better the interaction of all mechanisms of fat texture perception, it seems important to study the behavior of lipids in the mouth, specially spreading and persistence. Knowing how lipids interact, spread and retain on the oral surface will bring a valuable insight to better understand the differences in perception of aroma, texture and perhaps even taste. Therefore the trend is to find new in vivo instrumental methods that can reflect perceived oral texture (de Wijk et al., 2006a). Those methods are well applied in a range of semi solid foods. Tribological system was developed, that represents the tongue/palate contact and the conditions of oral cavity (Ranc et al., 2006b). It enables good measurements of friction coefficient, which relates well with the perception in the mouth. Very recently, Adams et al. (Adams et al., 2007) visualised in vivo food residues in the mouth with video rate endoscopy set up to investigate the food

behavior in oral cavity. Another study (Prinz et al., 2006b) related the turbidity of oral water rinses with the sensory attributes creamy, fatty, sticky and airy, for a series of dairy desserts varying in fat content between 0 and 15%. However, this study did not address the compositional changes of the lipid deposition and did not reveal any quantitative data on the distribution and retention of the food compounds on oral surfaces. Another study (de Jongh et al., 2006) evaluated semiquantitatively the lipid deposition by taking and evaluating swabs from the tongue with ATR FT-IR spectroscopy. Methods were developed to quantify distribution, deposition and retention of lipids on the oral surface upon ingestion (Pivk et al. 2008a). All these methods try to understand what in the mouth is happening in order to bring information on the mechanism of texture perception. Three different approaches were assessed to measure lipid deposition of the oral cavity: mouth rinse method, fluorescent probe method, filter paper method (Pivk et al. 2008a). The fluorescent probe method is a fast and simple method that showed the best properties for the quantification of lipid deposition. The thickness of lipid deposition on the tongue is patchy and it is increasing with increasing amount of oil until the saturation level. We have also observed higher thickness of lipid deposition on back and central area of the tongue, than on lateral area or palate (Pivk et al. 2008b). The samples were evaluated by a trained panel for three texture attributes: fatty film, lubricating film and sticky film. The intensity of "fatty" and "lubricating" film was increasing with oil volume (Pivk et al. 2008b). Significant difference was perceived among the highest and the lowest pure oil volume. Study indicated a relation between the lipid deposition on oral surface, especially on the tongue, and the sensory perception. Therefore, a direct measure of undisrupted residue of food components on oral surface will provide valuable information and contribute to the understanding of the behavior of food components in the mouth and their influence on perception. The modification of mouth rinse method quantified the oral coating after solid cheese consumption (Repoux et al. 2012).

Conclusions

Lipids as structural component greatly influence the structure of food. For example higher lipid content is reflected by lower friction, higher lubrication, higher viscosity, higher elastic properties, lower density and retarded melting. Via all these properties the lipids make significant contributions to the sensory perception. Texture perception occurs during first bite, mastication, swallowing and after swallowing.

Saliva contains many proteins, however only a few of them are known and their function well understood. Many are present in minor quantities and their role in oral food processing is yet unknown. Proteins are amphiphilic molecules, which have a tendency to go to the interface of two immiscible fluids. Small amounts of lipids present in saliva are incorporated mainly in the high molecular weight glycoproteins. Therefore salivary proteins might facilitate distribution of food lipids in the mouth. In fact, bovine submaxillary gland mucin was able, as an emulsifier, to stabilize o/w emulsions. Also lysozyme was shown to contribute to better foaming and emulsifying properties. However, conditions to obtain this in vitro were different than those in the mouth, so the remaining question is whether these proteins also are able to disperse food lipids in the mouth. Saliva also contains some proteins, which have known interfacial activity, like lingual lipase and some which transport lipophilic molecules, like lipocalins, VEG protein, and fatty acid transporter. It remains to be found out how important these molecules are in dispersing lipids in the mouth.

Little is known about the formation of mouth coating. There is no information on its structure, possible interactions or mechanisms which might contribute to its formation. In addition, several in-mouth parameters like temperature, shear rate, yield stress, oral surfaces, saliva flow and composition might significantly change physical properties of a lipid introduced in the mouth and therefore impact the perception. Lubrication might be one of the ways to perceive lipids. One study examined the influence of saliva addition on lubrication in non-mouth conditions and showed that mucins in saliva do not seem to influence lubrication, but more investigation is needed. Saliva changes the viscosity of liquids. Viscosity increases due to interaction between saliva and certain compounds in liquid or decreases due to dilution. Turbulent flow also increases perceived viscosity. Addition of saliva decreases the tendency of fluids to exhibit turbulent flow and hence viscosity. This might possibly be due to salivary glycoproteins.

Food emulsion flocculation was shown after mixing with saliva. There was no significant difference observed for saliva from different persons and for different types of emulsifiers. However, the overall appearance, i.e. the size of the lipid droplet aggregates and speed of aggregation, varied among the individual saliva samples. Mucins were shown to impact aggregation, but not alone, the contribution of other salivary proteins or even bacteria is also important. Depending on the charge of the emulsifier, the food emulsion adsorbed onto the mucin film. The adsorbed emulsions were more likely to lead to rough mouth feel. This effect was seen only at lower lipid levels. Adsorption is governed by the strength of the electrostatic forces that can be controlled through modification of the ionic environment and the pH of the system. The energy barrier produced by interfacial tension prevents one liquid from becoming emulsified into another. To form an emulsion, surface free energy must be lowered by adding a third component that seeks the interface. Some compounds in saliva, like mucins, lipocalins, lysozyme, lactoferrin and secretory IgA, lower the surface tension, however up to now how they act on the o/w interface has not been exploited. This might help to understand some mechanisms which occur during mouth coating.

Important findings that have not been so far clearly determined are different texture perception thresholds. We know we can perceive differences in viscosity, however it is not known what difference in viscosity is perceived in different viscosity ranges. The same would have to be done for other factors which are considered important in fat perception, such as viscosity, density, elasticity, lubrication, specific heat.

To achieve suitable mouth effect many processing inventions took place, like low-fat ice-cream, snack bars, yogurts… At the same time fat mimetic concepts are entering food production. The predictions about future obesity problems will drive development of this field of application. However, it is clear that without comprehensive understanding in the oral

cavity, we will not be able to help food designers develop products which will be acceptable to the consumer.

Lipids are an important part of food and many sensory perceptions are thought to be dependent on the oral coating. Saliva seems to be a potent and interesting contributor in the interaction with lipids and the formation of oral coating. Since thus far little research has been performed in this field, it would be of greatest interest to pursue this line of research to potentially answer the question how much fat is minimally needed not to compromise in fat perception.

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