Oral Coatings:

A Study on the Formation, Clearance and Perception

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Thesis

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"De hoogste vorm van geluk is een leven dat wordt gekenmerkt
door een zeker mate van zotheid"
Erasmus of Rotterdam

Para a minha mãe

Abstract

Introduction

Oral coatings are residues of food and beverages that coat the oral mucosa after consumption. Oral coatings are one of the factors influencing lubrication properties in mouth, taste and aroma perception. Although it is known that oral coatings can influence sensory perception, the understanding of the chemical composition and physical properties of oral coatings in relation to sensory perception is limited. The aim of this thesis is to understand which factors influence the composition, formation and clearance of oral coatings and their sensory perception.

Method

This thesis consisted of studies of oil coatings deposited on the tongue formed by oil droplets of oil/water (o/w) emulsions and protein coatings formed by the aqueous phase of o/w emulsions. The effect of oil content, protein content, protein in-mouth behavior and presence of thickener on the formation and clearance dynamics of oral coatings was investigated. A calibration method for *in vivo* fluorescence measurements to represent conditions occurring in mouth was developed to quantify oil oral coatings. Protein content of oral coatings was quantified using cotton swabs to collect protein oral coatings and subsequently quantifying protein concentration of the coating (mass protein/mass coating) with the Lowry method. Progressive sensory profiling was used to assess sensory perception of coatings over time. The effect of oral coatings on subsequent sweetness perception was studied by first coating the tongues of participants with o/w emulsions, and subsequently providing sucrose solutions and evaluating sweetness intensity.

Results

Oral coatings are formed within the first seconds of contact with the oral mucosa. These fast dynamics were observed for formation of oil coatings by liquid o/w emulsions and semi-solid emulsion-filled gels as well as for formation of protein coatings by the aqueous phase of o/w emulsions.

Oil coatings consisted of individual oil droplets deposited on the tongue surface rather than a continuous oil film. Increasing oil or protein content in the stimuli increased the oil or protein content deposited on the tongue. Proteins which display different in mouth behavior (proteins flocculating vs. proteins not

flocculating with salivary biopolymers) deposit similarly on the tongue. Presence of xanthan gum decreased the amount of oil and protein deposited on the tongue. This suggests that the formation dynamics of coatings is dependent on the availability of the ingredients to deposit between the voids of the papillae. In general, clearance of oil/fat coatings followed a similar tendency for all stimuli studied in this thesis. Most of the coating (> 60%) is cleared from the tongue in the first 45s. Exception occurred when oil coatings were formed by o/w emulsions stabilized by lysozyme.

Perception of oral coatings depends on the amount of oil and protein deposited on the tongue, the type of protein used (proteins which flocculate lead to high astringency) and the presence of thickener. Oral coatings formed by o/w emulsions do not influence subsequent sweetness perception of sucrose solutions.

Conclusions

Several factors were identified which affect after-feel and after-taste of oral oil and protein coatings, such as protein and oil content, protein type and lubrication in-mouth. Oral coatings formed by o/w emulsions do not influence subsequent sweetness perception since the amount of oil deposited on the tongue is not sufficient to form a hydrophobic barrier to limit the accessibility of sucrose to the taste buds.

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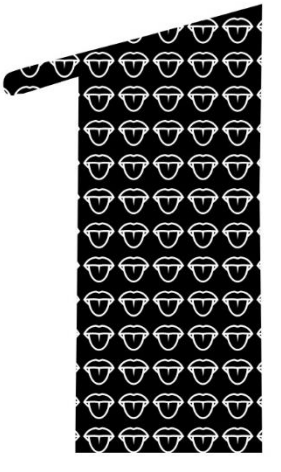

Consumers have been encouraged to consume less fat due to health and diet concerns. Therefore, it is necessary for food industry to produce low-fat products with the same organoleptic properties as the full-fat product. However, it remains a great challenge to mimic the rheological and sensory properties of full-fat products. Often low-fat products are perceived as less creamy or less tasty. To understand the dynamics of sensory perception of food products, the structural transitions which occur during oral processing need to be known.

For solid and semi-solid foods, the first and one of the most important factors of food dissociation is mastication. Mastication changes texture perception of foods. The action of the teeth create a force which disrupts the food matrix and releases the tastants and aromas into the oral cavity (Foegeding *et al.*,2011, Stieger and van de Velde, 2013). The release of tastants into the mouth and the migration of tastants to the taste buds triggers taste perception of the food product. Saliva is incorporated into the broken down fragments of the food creating a suitable bolus safe for swallowing (Chen and Stokes, 2012). After swallowing, remains of food, oral coatings, stick at the oral mucosa creating a lingering taste and affecting lubrication in mouth (de Wijk *et al.*,2006). For liquid foods, the effect of mastication on structural breakdown is often negligible. Nevertheless, the contact of liquids with the oral mucosa and saliva, and the oral coatings prevenient are of extreme importance for sensory perception.

1.1 Overall Aim and Approach

The aim of this thesis is the study of the deposition and clearance dynamics of oral coatings, their effect on mouth-feel and after-feel, and the role of oral coatings on subsequent taste perception. Oil/water (o/w) emulsions are used as model foods throughout the thesis as their physical-chemical properties can be controlled and o/w emulsions are considered to be representative models for beverages such as milk or drinking yoghurt.

The diagram below illustrates the approach and research questions of this thesis and will guide the reader throughout the introduction.

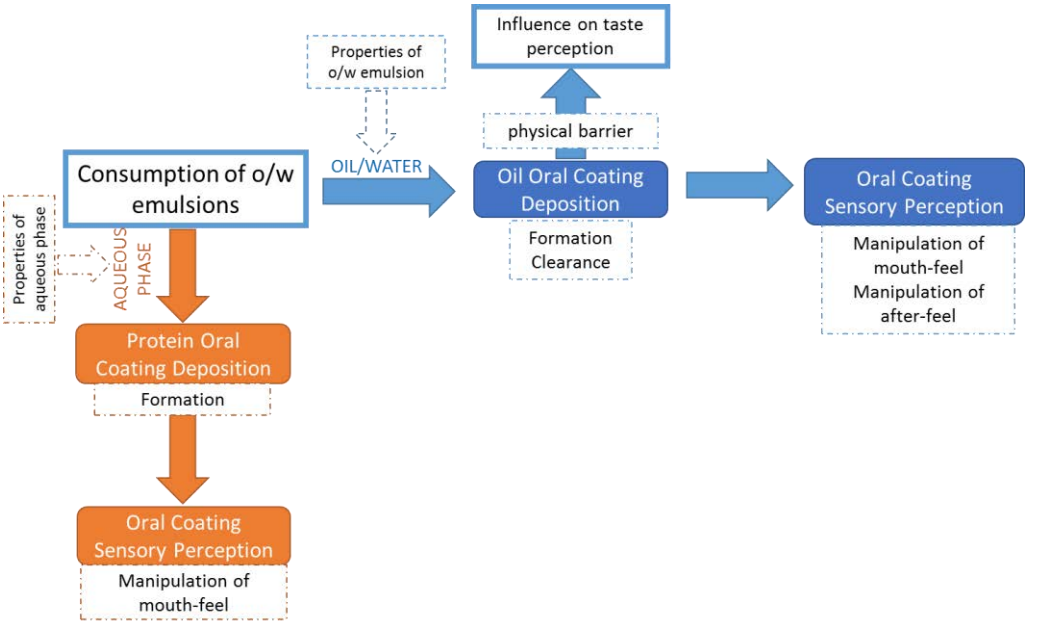

Figure 1.1. Study scope of the thesis. Dotted lines indicate the research questions of this thesis. Solid lines indicate studies described in literature.

1.2 Methodologies to Quantify Oral Coatings

The first step of the thesis was to develop reliable methods to quantify oral coatings deposited on the tongue.

Although it is well known that oral coatings can influence sensory perception, there is little information available on the chemical composition and physical properties of oral coatings. A small number of techniques to quantify oral coatings was described by literature.

A method to quantify the lipid content of the oral coating is by rinsing the oral cavity and subsequently determining the turbidity of the spat out solution as a measure of lipid content. Prinz *et al.*,(1996) used custards varying in fat and starch content and determined the coating's lipid content of two subsequent spat-out

solutions. The first spat out (representing the top layer of the coating) related to the sample's viscosity and varied with both the fat and starch content. The second spat out (bottom layer of coating) varied only with the fat content. These variations were verified through variations on the consumed samples.

Prinz *et al.*, (1996) suggested that the composition of the oral coating is non-homogeneous (Prinz *et al.*,1996). Using the same methodology, de Wijk *et al.* (2009), investigated the role of salivary enzyme amylase on the clearance rate of fat containing oral coatings, by comparing enzymatic degradable starch-based custards with non-degradable sodium carboxyl methycellulose (CMC) custards. The clearance rates of starch-based custards coatings were faster, compared to CMC based custards coatings, indicating a role of salivary amylase in the clearance of starch-based foods (de Wijk, 2009). This method is fast and easy to apply, but it is possible that the rinsing of the coating is incomplete, and some of the fat remains attached to the oral surface. Turbidity is taken as a measure of lipid content, but this might be an oversimplification, as turbidity of the spat out solutions depends on fat content, droplet size, solubility, and possible the presence of other components that scatter light. Furthermore, this method is not able to quantify compositional changes of lipid deposition.

de Jongh and Janssen (2007) used attenuated total reflection IR (ATR-IR) spectroscopic analysis to analyze swabs containing oral coatings taken from distinct parts of the oral cavity, over time. With this approach, the fat, protein and carbohydrate contents of oral coatings of three dressings (40% oil, 10-14% protein and either starch, xanthan or a mixture of these two thickeners) could be determined. In contrast to the turbidity method, ATR-IR provides information about the chemical composition of the swabs. The relative contributions of oil versus protein/carbohydrate were determined by taking the integrals of both contributions from the ATR spectrum (de Jongh and Janssen, 2007). However, this method is not able to measure the density of oral coatings and, as this is an

ex-vivo technique, its efficiency is limited to an extraction step to collect the oral coating.

Pivk *et al.*,(2008a) used filter papers to extract oral coatings from the tongue formed by medium-chain triglycerides (MCT oil) samples. The filter papers were pressed on the tongue surface and subsequently the lipids were extracted from the papers with a mixture of chloroform/methanol. The lipids contained curcumin as a hydrophobic, fluorescent dye. The fluorescence intensity of the extracts was measured to quantify lipid content. It was not possible to recover all the lipids from the tongue surface using this method. Therefore, Pivk *et al.*, (2008a) developed an *in vivo* method to characterize the thickness of oral coating by fluorescence measurements. Fluorescence was measured with a fluorescence spectrometer coupled with a remote read fiber-optic probe fitted with a tip for measurement on surfaces, such as the tongue. In order to calibrate the measured fluorescence intensity with the thickness of the lipid layer, different volumes of curcumin containing a continuous and homogeneous layer of oil were spread on a Petri dish at room temperature to obtain oil layers varying in thickness (Pivk *et al.*,2008a). Using the same method Pivk *et al.*, (2008b), showed that the thickness of the lipid deposition on the tongue depends on the position on the tongue, with thicker coatings at the back of the tongue and a thinner coatings on the lateral area of the tongue. With increasing oil volume (8 mL), the thickness of the coatings increased up to 50μm and did not increase further upon addition of more oil. Differences on MCT intake created thicker depositions of oil in mouth, and increased the perception of the attributes "fatty film" and "lubricating film". Changes of 25μm in thickness of the oil coating resulted in significantly differences in sensory perception. The retention of lipids showed an exponential decrease in thickness over time, measured on three time points (Pivk et al, 2008b).

A recent study applied the fluorescence method to compare the thickness of oral coatings formed after consumption of dispersions of MCT oil in water with oral coatings formed after consumption of pure MCT oil. Dispersions of MCT oil in water had a lower thickness of lipid deposition in mouth and created a less

pronounced mouthfeel perception of "fatty" and "lubricating film" attributes compared with pure MCT oils with the same amount of oil. It was hypothesized that the addition of water could be responsible for a better dispersion of the oil in the saliva, which would reduce the availability of the oil to deposit on the oral surfaces (Kupirovic *et al.*,2012).

The *in vivo* fluorescence method provides a direct measure of oral coatings thickness, without damaging any of its components. This method allows the study of the spatial variation of the coating on the tongue over time. Due to the morphology of the tongue it is important to study oral coatings spatial variation. Taste detection occurs in taste-receptor cells. Taste receptor cells are transmembrane proteins which are clustered into taste buds. Taste buds are present in different papillae on the tongue and palate epithelium. The tongue contains four different papillae: filiform, fungiform, foliate and circumvallate papillae. Filiform papillae do not contain taste buds, but are likely involved in texture perception as mechanoreceptors. The remaining three types of papillae contain taste buds. Each type of papillae is found in different places on the tongue (figure 1.2), (Chandrashekar *et al.*,2006, Kullaa-Mikkonen *et al.*,1987).

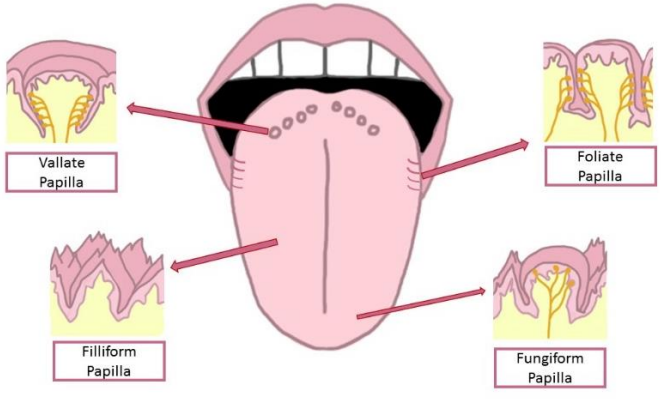

Figure 1.2. Schematic representation of the distribution of the papillae over the tongue surface.

As the tongue morphology is so complex, it is likely that oral coatings deposit differently depending on the different structures present on the tongue. As such,

one of the aims of **chapter 2** and **3** was to study how oil oral coatings deposit and clear on different parts of the tongue.

The first step to use *in vivo* fluorescence is to correlate fluorescence intensity with coating deposition. The calibration of fluorescence *vs.* coating thickness is a crucial step. Pivk *et al.*,(2008b) used the surface of a Petri dish which is different from the surface of a human tongue. The human tongue, as already explained is rough and covered with papillae. The surface properties might influence the fluorescence intensity of the coatings. Additionally, it is known that fluorescence intensity of curcumin depends on temperature. Therefore, the calibration should reflect the temperature of the coating in the oral cavity. Further, most of the mentioned studies have worked with pure oils. However, most beverages consist of oil in water (o/w) emulsion that are stable under in mouth conditions, and have a very different structure from pure oil. When the o/w emulsions form an oral deposit, it is likely that the coating consists of discrete oil droplets adhering to the tongue surface, and not a continuous film of oil.

The aim of **chapter 2** laid on the development of an appropriate calibration method for *in vivo* fluorescence measurements which represents the *in vivo* conditions occurring in mouth. Pig's tongue samples at body temperature were used on the calibration as pigs are omnivores, and have a similar nutrition as humans. Furthermore, as on the human tongue, the anterior part of pig's tongue mucosa is covered by fungiform papillae scattered between filiform papillae. Filiform papillae of the human and pig tongues have the same shape, *i.e.* each of it bears many secondary papillary projections. Further, **chapter 2** shifted the study direction from pure oils to o/w emulsions as more representative and relevant models of commercial beverages.

In vivo fluorescence was applied to study the oil deposition on the tongue of o/w emulsions varying in oil content, and to establish psychophysical relationships between oil coatings formed on the tongue and after-feel perception. Results on **chapter** 2, showed that *in vivo* fluorescence was a reliable method to quantify oil

fraction (mass of oil/ area tongue) deposited on the tongue. For this reason *in vivo* fluorescence was further used to study the formation and clearance of oil/fat oral coatings in **chapter 3, 4** and **chapter 5**.

As oral coatings are directly related to the consumption of foods, proteins can also deposit on the tongue surface and consequently influence sensory perception. The aqueous phase of o/w emulsions, which was hypothesized to create protein coatings was also investigated through *in vivo* fluorescence. Unfortunately, this did not prove to be successful, thus another method had to be developed in **chapter 6**. The aim of **chapter 6** was the development of a method to quantify protein content in the oral coatings, and determine the influence of protein content, in-mouth protein behavior and presence of thickeners on the formation dynamics of protein oral coatings and sensory perception of protein solutions.

1.3 Properties Influencing Formation and Clearance of Oral coatings

1.3.1 Food Emulsions

An emulsion is a mixture of at least two immiscible liquids in which one liquid is in the form of droplets dispersed in the continuous, liquid phase. An interfacial layer composed of surface active agents is present between the two liquids, such as proteins or emulsifiers. In foods, lipids are often present in the form of emulsions. Common food emulsions include milk, drinking yoghurts, mayonnaises and butter.

Water and oil can form different types of emulsions depending on which liquid is the dispersed phase. When oil is dispersed in a continuous aqueous phase the emulsions are termed oil-in-water (o/w) emulsions, such as milk or mayonnaise. When water is dispersed in a continuous oil phase the emulsions are termed water-in-oil (w/o) emulsions, such as butter.

1.3.2 Stability of Food Emulsions: Flocculation and Coalescence

Emulsions are thermodynamically unstable systems as the free energy of mixing (ΔG_{mix}) is always positive due to the large interfacial area between the oil and the

aqueous phase (Walstra, 1996). Stability of emulsions relates to the time period for which an emulsion keeps its physical-chemical properties unchanged. Stability of emulsions depends greatly on the type and concentration of the dispersed phase, the continuous phase and most importantly, the properties of the interfacial layer. Further, pH, viscosity and physical/chemical conditions have a large impact on the stability of the emulsion (McClements, 2005).

Emulsion´s instability can be promoted due to physical or chemical factors. Chemical instabilities relate to changes in the molecules such as oxidation of fat or hydrolysis of fat or proteins. Physical instabilities relate to changes of the structure or distribution of the droplets in the aqueous phase such as creaming, flocculation or coalescence. Physical instabilities can occur to emulsions in the oral cavity as a consequence of shear forces occurring during oral processing, changes in pH occurring in mouth and as a consequence of interactions of the emulsion droplets with salivary biopolymers. This thesis focuses on flocculation and coalescence of emulsion droplets in the oral cavity (**figure 1.3**).

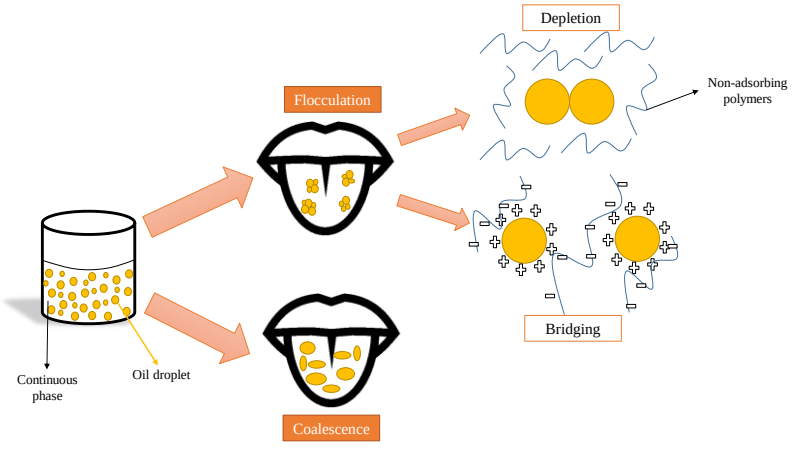

Figure 1.3. Schematic representation of emulsion instabilities investigated in this study.

Coalescence (**figure 1.3**) is the irreversible process of two or more smaller droplets merging to form one larger droplet. Coalescence can finally lead to the formation of a macroscopically phase separated system. Food emulsions are usually designed to be stable against coalescence by using emulsifiers. Under in-mouth conditions, the emulsion stability may be affected due to shear forces of the tongue pressing against the palate during consumption. The effect of protein-poor against protein-rich o/w emulsions on the adhesion and spreading of fat on the tongue was previously reported. It was found that o/w emulsions stabilized with low concentrations of protein had a stronger adhesion to the tongue surface compared to o/w emulsions stabilized with high concentrations of protein. It was hypothesized that this was due to differences in stability of the interfacial layer of the emulsion against rupture by shear leading to different in-mouth behavior revealing different levels of coalescence of oil droplets (Dresselhuis *et al.*,2008). The coalescence in mouth was shown to be more probable when the emulsions were less stable *i.e.* stabilised by a small amount of emulsifier (Dresselhuis *et al.*,2008).

For this thesis a correct descriptor for oil coatings is of extreme importance. For a correct descriptor, the knowledge on the structure of the coating on the tongue (*i.e.*, homogeneous layer of oil or independent oil droplets) is essential. As such, **chapter 2** analyzed, with CLSM images, the stability against coalescence of a 20% o/w emulsion (maximum oil content used in this thesis) on top of a pig's tongue by mimicking in-mouth conditions. This *in vitro* experiment allowed to draw conclusions over the oil coating structure and define a new descriptor: oil fraction (mass of oil/area of tongue).

Literature on oral coatings formed by semi-solids and solids is scarce and evidence for an increase of fat in oral coatings due to fat release and coalescence during breakdown of emulsion-filled gels is not available. To our knowledge, Repoux *et al.* (2012) were the first and the only study that focused on oral fat coatings formed from solid foods (cheese) (Repoux *et al.*,2012). In the mentioned study, the oral coatings were collected by asking the participants to rinse their

mouth with water after masticating cheese, which might have made the collection of the coating incomplete. Therefore, it is desirable to quantify oral coatings directly in mouth by *in vivo* fluorescence spectroscopy without an extraction/collection step in between. Further, previous studies have suggested that the release of fat from emulsion-filled gels (models for semi-solid and solid foods) and coalescence of fat droplets under mouth-mimicking in vitro conditions is related to a decrease in friction, leading to an increase in perception of fat-related sensory attributes (Dresselhuis *et al.*,2007; Sala *et al.*,2007b; Liu *et al.*,2015). However, these conclusions were never proved by *in vivo* trials. As such the aim of **chapter 5** was to investigate the influence of oral processing and fat droplet characteristics of emulsion-filled gels on the formation and clearance of fat deposition on the tongue in relation to mouthfeel and after-feel sensory perception. Two emulsifiers were used: whey protein isolate and Tween 20. Whey protein isolate comprises a mixture of globular proteins: β-lactoglobuline ~55%, α-lactalbumine ~24%, serum albumin ~5% and immunoglobuline. Whey proteins have an isoelectric point of ~4.5, forming negatively charged emulsion droplets at a neutral pH. Tween 20 is a non-ionic polysorbate surfactant. It was hypothesized that oral processing of emulsion-filled gels stabilized with Tween 20 would create coalescence in mouth and thus higher fat fraction on the tongue, compared with emulsion-filled gels stabilized with whey protein isolate. These two emulsifiers were used in the study in order to create bound (WPI) and unbound (Tween 20) emulsion droplets in gelatine gels.

Flocculation (**figure 1.3**) occurs when droplets associate into reversible aggregates due to unbalanced attractive and repulsive forces (Dalgleish, 1997). Flocculation can be of two types: depletion and bridging flocculation.

Depletion flocculation occurs due to the presence of a solution of non-adsorbing polymers. These polymers induce an osmotic pressure gradient in the continuous phase surrounding the droplets, promoting their association and causing flocculation (Dickinson *et al.*,1997a). The flocculation properties of o/w emulsions

stabilized with Na-caseinate were previously shown to be comparable to micelle induced depletion in surfactant-based systems. As the unadsorbed protein concentration is increased in the continuous phase of the o/w emulsion the flocculation is also expected to increase. This flocculation is weak, reversible and occurs before oral processing. The flocculation is likely to be disrupted by in-mouth shear and dilution with saliva.

Bridging flocculation occurs when a high molecular weight polymer adsorbs to two or more emulsion droplets forming bridges between different droplets (Dickinson *et al.*,1997b). For instance, bridging flocculation occurs when lysozyme is in contact with salivary biopolymers. Lysozyme forms complexes with salivary biopolymers by electrostatic interactions between the positively charged lysozyme and the negatively charged salivary biopolymers (Silletti *et al.*,2007). Bridging flocculation is irreversible and was reported to affect after-feel and after-taste perception of model foods (Vingerhoeds *et al.*,2009). This effect was suggested to be due to the in in-mouth behavior caused by the proteins. The saliva induced flocculation of the o/w emulsion droplets stabilised by lysozyme reduces the lubrication of saliva and increases friction in mouth which leads to astringent and rough after-feel (Vingerhoeds *et al.*,2009). In these studies the quantification of oral coatings was made *ex vivo*. Further, the mechanisms on how different proteins, which behave differently in in-mouth, influence the deposition and clearance of oil oral coatings are still not completely understood. As such, **chapter 3** aimed at investigating the effect of different protein type and protein content on the clearance of oral oil coatings and after-feel sensory perception. The clearance of oral oil coatings from the tongue surface was determined using *in vivo* fluorescence measurements. The after-feel perception was determined using sensory progressive profiling. To study the influence of protein emulsifier type on oil coatings, o/w emulsions stabilized with proteins differing in flocculation behavior when mixed with saliva were prepared: Sodium Caseinate (Na-Caseinate) and lysozyme. Protein content was also studied as it was hypothesized to contribute to the clearance of the oil coatings.

Na-Caseinate is a milk protein which is commonly used as emulsifier on o/w emulsions. The distinct hydrophobic and hydrophilic regions of the caseins allow a fast adsorption to the oil-water interface. The oil droplets are well stabilized through a combination of steric and electrostatic interactions (Dickinson *et al.*,1997a). Nevertheless, when in excess Na-caseinates form aggregates (sub-micelles of ~2.5 x 10^5 Da) in the aqueous phase due to hydrophobic associations (Dickinson *et al.*,1997b). Na-caseinate has an isolectric point of ~4.6, forming negatively charged emulsions at neutral pH. Na-caseinate was chosen as an emulsifier in the studies (chapter 2, 3, and 4) as it was hypothesized to not interact with saliva during the emulsions oral processing.

Lysozyme is a sweet tasting protein composed of 129 amino acid residues. Lysozyme is a globular protein with an isoelectric point of ~10.5, forming positively charged emulsion droplets at neutral pH. Lysozyme was chosen as an emulsifier in the study (chapter 3) as it is known to agglomerate with salivary biopolymers through electrostatic interactions (Silletti *et al.*,2007).

In contrast to oil oral coatings, scarce literature exists on the dynamics of formation and clearance of protein oral coatings. As such, the aim of **chapter 6** was to determine the influence of protein content and in-mouth protein behavior on the dynamics formation of protein oral coatings and sensory perception of coatings from protein solutions. To this end, and to be able to create parallels with oil oral coatings, the same proteins studied on **chapter 3** were studied on **chapter 6**: Na-caseinate and lysozyme. To identify the individual importance of the protein on the oral coatings and to minimize the interference with other macronutrients, this study used aqueous solutions of proteins.

1.3.3 Viscosity of Food Emulsions (addition of thickeners)

Texture perception of foods is affected by lubrication through the different oral processing stages. Rubbing and squeezing the food between tongue and palate are important for the detection of sensations such creaminess or slipperiness where

the food can act as a lubricant and reduce the friction, thus increasing lubrication, between the two interacting surfaces (Prakash *et al.*,2013). Lubrication in mouth can be influenced by the addition of thickeners in foods. Van Aken *et al.*,(2011) found that the o/w emulsions after-feel perception of attributes such as of coating after-feel, fatty and slippery mouth-feel increased when arabic gum was added to o/w emulsions. This demonstrates that thickeners contribute to the sensory perception of fat related attributes in o/w emulsions. Vingerhoeds *et al.*, (2009) hypothesized that a thickener can form a layer similar to oil on oral surfaces or imitate an oil layer due to increased viscosity in mouth. It was found that addition of guar gum to o/w emulsions resulted in a slightly reduced oil retention on the tongue surface (Vingergoeds *et al.*, 2009). de Jongh and Janssen (2007) found no differences in oil retention on the tongue between dressings differing in type of thickeners (starch, xanthan and a mixture of the two) (de Jong and Janssen, 2007). Although the mentioned studies have provided relations between the effects of thickeners on oral coatings perception, the quantification of the effect of the thickener on oil and protein coatings on perception is not known. Thus, one of the aims of **chapter 3** was the study of the effect of viscosity of o/w emulsions on the clearance of oral oil coatings and after-feel sensory perception. On the other hand, and as most of the studies focused on oil oral coatings, one of the aims of **chapter 6** was to study the effect of thickener on the aqueous phase of o/w emulsions, on the dynamics formation of protein oral coatings and sensory perception of protein solutions. For both studies, the thickener used was xanthan-gum.

1.4 Influence of Oral Coatings on Sensory Perception

1.4.1 Mouth-feel and After-feel of Oral Coatings

As mentioned on the previous subchapter, oral coatings are one of the factors influencing lubrication properties in mouth, and after-feel perception. The influence of oral coatings on lubrication in mouth, was described by de Wijk *et al.*,(2005). It was suggested that fat containing oral coatings lubricate the

movement of the food bolus on the oral tissue leading to lower intensities of perceived dryness and roughness and higher intensities of creaminess of custards. de Wijk *et al.*,(2005) provided evidence for this hypothesis, showing that astringency of custards was related to high in mouth friction due to low amounts of oral coating and creaminess to low in mouth friction due to higher amounts of oral coating. In mouth friction is also affected by viscosity of the samples consumed. Two common ways in which viscosity can be modulated is through the addition of thickeners (subchapter 1.3.3), or by increasing oil content of the o/w emulsion. Van Aken *et al.*, (2011) found that creamy and coating after-feel and fatty and slippery mouthfeel increases with increasing oil content of emulsions. Similarly, Pivk *et al.*, (2008) also found that oral coatings with higher deposition of oil on the tongue lead to increased perception of fatty film and lubricating film.
The difference in in-mouth behavior caused by the different proteins also causes differences in the perception of oral coatings. As lysozyme forms complexes with salivary proteins the saliva induced flocculation of the o/w emulsion droplets reduces the lubrication of saliva and increases friction in mouth which leads to astringent and rough after-feel (Vingerhoeds *et al.*,2009).
The oral coating´s mouth-feel and after-feel of a food product can likely lead to the acceptance or rejection of a product. The direct link of the amount of coating on the tongue and its´ influence on perception is still not fully drawn. For this reason one of the most important aims of **chapter 2, 3, 5** and **6** was to link the amount of oral coating on the tongue with the mouth-feel and after-feel perception of the coating.

1.4.2 Oral Coatings Influence on Subsequent Taste Perception

Oral coatings have been shown to influence taste and aroma perception. Madrigal-Galan and Heymann (2006) observed a decrease in intensity of several wine sensory attributes, such as astringency, bell pepper, and oak flavor, when the wine was evaluated after eating cheeses. It was hypothesized that the decrease in intensity might be caused by an oral coating of fat in the oral cavity formed by the

cheese (Madrigal-Galan and Heymann, 2006). Lynch *et al.*, (1993) investigated the effect of oral coatings formed by different types of oil on the subsequent perception of taste intensity of gelatin gels with added NaCl, sucrose, quinine sulphate or citric acid, using time-intensity methodologies. The oil coating reduced the maximum and overall taste intensity of the subsequent taste stimuli. It was suggested that the oral fat coating influences taste perception by modification of the partitioning of specific compounds between the food, saliva and taste receptors, or by forming an hydrophobic fat layer creating a physical interference for the hydrophilic tastant to access the taste receptors, leading to a reduction in taste intensity (Lynch *et al.*,1993).

Literature suggested that oil coatings form a physical barrier which would prevent tastants to pass through, and thus decrease the subsequent taste perception. The aim of **chapter 4** was to investigate the existence of a physical barrier formed by o/w emulsions and to measure the effect of oil oral coatings on subsequent sweetness perception, using *in vivo* fluorescence measurements to quantify the oil deposited on the tongue and a trained panel to evaluate subsequent sweetness perception. Further, as the previous chapters had been focusing mainly on the dynamics of clearance of the oil oral coating, **chapter 4** focused on the dynamics of formation of oil oral coatings.

1.5 Taste and Texture Sensitivity

One of the most common methods to quantify taste sensitivity is by determination of sensory difference thresholds or Just Noticeable Differences (JND´s). JND's are defined as the minimal difference that can be perceived between two stimuli (Lawless and Heymann, 2010). JND´s are important for food industry. Food technologists can adjust the ingredients of a product in a way that the consumer may or may not perceive the difference. In other words, when the aim is for the consumers to notice a change in the product (*e.g.*, when the consumer prefers a sweeter product), the JND for sweetness should be exceeded between products. If the opposite is required, (*e.g.*, when the aim is to lower the sugar-concentration

but keep the sweetness unchanged), the JND should not be exceeded between products. In the latter, the perception of the product remains the same, but the amount of sugar can be lower.

JND´s are usually determined using the method of constant stimuli. The method of constant stimuli compares a stimulus always to a constant reference stimulus which is the middle point in a series of comparisons. The relation between JND's and the reference stimulus results in the Weber fraction (K) (**Figure 1.4**). The Weber fraction is an index of the sensitivity of the sensory system to detect changes of a certain stimulus (Lawless and Heymann, 2010).

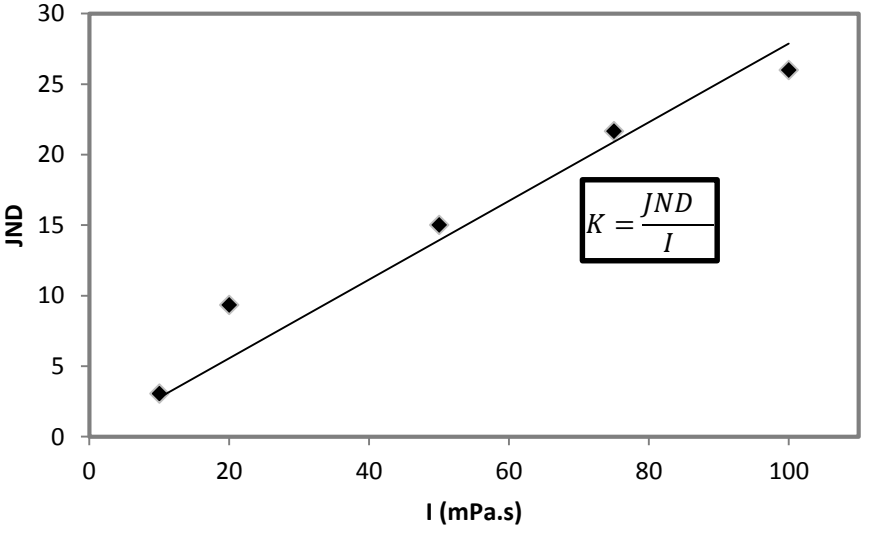

Figure 1.4. Schematic determination of the Weber fraction (K).

JND´s and Weber fraction have been extensively studied across taste modalities (Schutz and Pilgrim 1957; Stone and Bosley 1965; McBride 1983; Goldstein 2010; Orellana-Escobedo *et al*.,2012). Weber fractions for sucrose vary from K=0.13 to K=0.17 (Schutz and Pilgrim 1957, McBride (1983)). Within the same basic taste (sweetness) K´s can have different values depending on the molecule studied. For instance, high intensity sweeteners have thresholds which can be 100 or 1000 folds lower than the previous mentioned. For other modalities, Weber fractions

for saltiness, sourness and bitterness obtained were reported to be K=0.15, 0.22 and 0.30, respectively (Schutz and Pilgrim 1957).

Texture sensitivity is much less understood than taste sensitivity. In contrast to the studies on JND's of taste, little is known about JND's for texture perception of foods and beverages. JND's for creaminess perception when the apparent viscosity of dairy-based emulsions varied was recently reported (K=0.20) (Zahn *et al.*, 2013). Rohm and Raaber (1992) studied the JND's and Weber fraction for kinesthetic firmness perception and spreadability perception of edible fats by asking subjects to cut and spread different spreads with a knife (Rohm and Raaber 1992). They found K=0.20 for firmness perception and K=0.27 for spreadability perception. Changing a single textural property of foods and beverages without modifying other sensory properties such as taste and flavour remains a technical challenge and often requires the use of model foods. This limitation is likely the reason why there are very limited numbers of studies focusing on the determination of JND's of texture perception despite its important contribution to the appreciation of beverages and foods.

One of the fundamental questions which arose during the studies was the influence of thickness perception on the sensory evaluation of oral coatings. As mentioned before, literature on texture sensitivity is rare. As such the aim of **chapter 7** was the determination of JND´s and Weber fraction of oral thickness perception of Newtonian model stimuli using the method of constant stimuli.

Physical and Sensory Characterization of Oral Coatings by Oil/Water Emulsions

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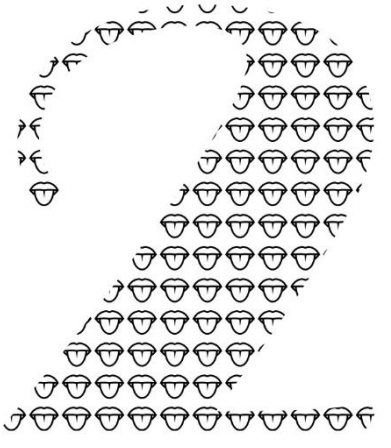

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Abstract

The physical and sensory properties of oil coatings on the tongue formed by five oil/water emulsions varying in oil content were investigated. Twenty subjects processed orally each emulsion for 30s in triplicate. *In vivo* fluorescence measurements at the front and back of the anterior tongue were made to quantify the oil fraction deposited at different time points. Calibration lines relating fluorescence intensity to oil fraction were determined using pigs tongues at 37.5°C to mimic oral conditions. The oil fraction on the tongue increased linearly with increasing oil content of the emulsions. Oil fraction deposited at the back of the anterior tongue was 1.5-2.0x larger than at the front. Intensity of sensory attributes describing after-feel perception was related to oil fraction by Weber-Fechner's law. This study uses *in vivo* fluorescence to study food behavior in mouth and unravel new insights in after-feel perception of emulsions.

Keywords: oral coating, tongue, oral processing, fraction of oil, fluorescence, curcumin, emulsion, sensory perception, mouth-feel sensation, after-feel perception

1. Introduction

Residues of foods and beverages that coat the oral mucosa after consumption are termed oral coatings. Only limited information is available on the chemical and physical properties of oral coatings. A method to quantify the lipid content of oral coatings has been proposed by rinsing the oral cavity after food ingestion and subsequently determining the turbidity of the spat out liquid (Prinz *et al.*, 2006, de Wijk *et al.*, 2009). de Jongh and Janssen (2007) used attenuated total reflection infrared spectroscopy (ATR-IR) to analyze the chemical composition of oral coatings taken with swabs from distinct parts of the oral cavity. This method allowed to determine the relative content of fat, protein and carbohydrate of oral coatings de Jongh and Janssen (2007). Pivk *et al.*, (2008a) developed an *in vivo* method to characterize the thickness of oral oil coatings on the tongue using fluorescence measurements. Fluorescence was quantified with a fluorescence spectrometer coupled with a remote read fiber-optic probe fitted with a tip for measurements on surfaces such as the tongue (Pivk *et al.*, 2008a). Pivk *et al.*, (2008 b) showed that thicker lipid coatings were formed at the back of the anterior tongue compared to the lateral tongue area (Pivk *et al.*, 2008b). The *in vivo* fluorescence method provides a direct measure of the thickness of oral coatings and allows to study the spatial variation of coatings on the tongue and to link instrumental measurements to sensory perception (Pivk *et al.*, 2008b). Pivk *et al.*, (2008a,b) used thickness of the coating as the physical parameter to describe oil coatings. Thickness suggests that a continuous, homogeneous film of oil is deposited on the tongue. That may be the case for orally processed oils as studied by Pivk *et al.*, (2008a,b). However, most beverages contain oil in water (o/w) emulsions and are stable under in mouth conditions. When o/w emulsions form an oral deposit, it is likely that the coating consists of individual oil droplets adhering to the tongue surface rather than a continuous, homogeneous oil film. Therefore, the mass of oil deposited on the tongue is a more descriptive parameter to characterize oral coatings formed by o/w emulsions rather than thickness.

The calibration of fluorescence intensity *vs.* coating deposition is a crucial step for the *in vivo* fluorescence method. Pivk *et al.*, (2008 a,b) calibrated the fluorescence intensity with the thickness of the oil coating by spreading different volumes of MCT oil containing curcumin, a hydrophobic, food grade fluorescent dye, in a Petri dish at room temperature to obtain oil coatings varying in thickness (Pivk *et al.*,2008a,b). It is known that fluorescence intensity depends on temperature. Therefore, the calibration temperature should reflect the temperature of the coating in the oral cavity. The surface properties used for the calibration can also influence the fluorescence intensity. Thus, the surface should be as similar to the human tongue as possible. The surface of a Petri dish is different from the surface of a human tongue which is rougher and covered with papillae. Pigs tongues are considered to be good models for human tongues (Dresselhuis *et al.*, 2008a, Kullaa-Mikkonen *et al.*, 1987). Pigs are omnivores and the anterior part of their tongues is covered by fungiform papillae scattered between filiform papillae similar to human tongues. Filiform papillae of the human and pigs tongues have similar shapes (Montavon *et al.*, 1991).

Oral coatings are one of the known factors that can influence after-feel, mouth-feel perception and taste. de Wijk *et al.*, (2006, 2003) demonstrated that fat containing oral coatings lubricate the movement of the food bolus leading to decreased roughness and increased creaminess perception (de Wijk *et al.*, 2006, 2003). Lynch *et al.*, (1993) showed that oral coatings formed by different oils reduce the subsequent perception of taste intensity of gels. It was suggested that oral fat coatings influence taste by modification of the partitioning of specific compounds between food, saliva and taste receptors or by forming a hydrophobic layer creating a physical barrier for hydrophilic tastants limiting access to taste receptors (Lynch *et al.*, 1993). Although it is well known that oral coatings can linger in the mouth after swallowing, little is known about the influence of oral coatings on after-feel sensory perception (mouth-feel perception occurring after swallowing). We hypothesize that psychophysical relationships can describe the

link between oil coatings lingering on the tongue after expectoration and after-feel perception.

This study is based on the method previously described by Pivk *et al.*, (2008a, b). The aims of this study were to (i) develop an appropriate calibration method for *in vivo* fluorescence measurements which represents closer the *in vivo* conditions occurring in mouth; (ii) apply the method with the new calibration using o/w emulsions varying in oil content; (iii) establish psychophysical relationships between oil coatings formed on the tongue and after-feel perception.

2. Materials and Methods

2.1 Materials

Sunflower oil (Euroshopper, purchased from local retailer), reverse osmosis water, sodium caseinate (Excellion sodium caseinate S, DMV International, The Netherlands) and curcumin (7% curcumin solution in propylene glycol and polysorbate, L-WS; Sensient, The Netherlands) were used. Pigs tongues were provided by VION Food Group (The Netherlands). All ingredients used were food grade.

2.2 Emulsion Preparation

Five o/w emulsions were prepared with an oil content of 1, 5, 10, 15 and 20% w/w. 3.2% w/w sodium caseinate was added to the water phase of all emulsions independent of oil content. The emulsions were pre-homogenized using an ultra turrax (IKA® RW 20 Digital) and homogenized (Niro-Soavi S.p.A. NS1001L2K) with pressures between 300-400 bar. The droplet size distribution was determined by light scattering (Mastersizer 2000, Malvern Instruments, Goffin Meyvis). The average diameter of emulsion droplets ($d_{3,2}$) is presented in Table 2.1. Curcumin solution was added immediately before consumption to each o/w emulsion (**Table 2.1**) until saturation which was verified through fluorescence

intensity measurements (data not shown). All samples were prepared in a food grade environment and stored at 4°C for a maximum of 2 days.

Table 2.1. Composition of all o/w emulsions used in this study and average diameter of emulsion oil droplets ($d_{3,2}$). All o/w emulsions contained 3.2 % w/w Na caseinate.

[Oil] $_{emulsion}$ (% w/w)	[Curcumin] (‰ v/w)	Oil droplet size $d_{3,2}$ ± Std. Error (µm)
1	0.027	1.16 ± 0.07
5	0.040	1.32 ± 0.12
10	0.070	1.37 ± 0.15
15	0.088	1.34 ± 0.16
20	0.105	1.17 ± 0.07

2.3 Pigs Tongues Preparation

Pigs tongues were prepared and preserved as previously described (Dresselhuis *et al.*, 2008a). The pigs tongues were collected immediately after slaughter and stored in a physiological salt solution for transportation. The tongues had a length of about 18 cm. Only the middle part of about 6-8 cm was used since the back part of the tongue has larger papillae compared to the human tongue and the front part was damaged during processing in the slaughter house. The pigs tongues were cut into 3 parts. The middle part was snap frozen in liquid nitrogen and stored at -80°C. The pigs tongues were thawed shortly before use in tap water. The pigs tongues were cut into pieces of 2x2 cm resulting in a tongue surface area of 4 cm2.

2.4 Confocal Laser Scanning Microscopy (CLSM): Microstructure of o/w emulsions on pigs tongues

The microstructure of the o/w emulsions on pigs tongue was analyzed by Confocal Laser Scanning Microscopy (TCS SP5 Confocal Laser Scanning Microscope, Leica Microsystems, Heidelberg, Germany). The objective lens used

was a dry HCPL apo 20x/0.70CS (zoom 2). A layer of human saliva was added to the pigs tongues. Images were collected under three experimental conditions: (i) plain pig's tongue with layer of human saliva without o/w emulsion, (ii) pig's tongue with layer of human saliva and 10% (w/w) o/w emulsion, (iii) pig's tongue with layer of human saliva and 10% (w/w) o/w emulsion after being rubbed with another piece of pig's tongue to simulate the rubbing of the human tongue against the palate during drinking. The emulsions were stained with an aqueous solution of 0.5% w/w Nile Blue.

2.5 Fluorescence Measurements and Calibration

Calibrations were made to correlate fluorescence intensity of curcumin in the o/w emulsion to oil content deposited on the tongue. For this purpose a single point fluorescence measurement (Fluorolog Instruments SA Inc, Jobin Yvon Spex) was used with an excitation wavelength of 440 nm, an emission wavelength of 495 nm with a slit width of 0.95 mm and a measurement time of 0.1s. A fluorescence remote read fiber optic probe was used to measure on tongue surfaces. A plastic ring (diameter 16.9 mm, height 5 mm) was attached to the end of the probe to ensure that the distance between the probe and the surface remained constant. The probe was put gently on the tongue to avoid deformation of the tongue surface. For all measurements the background (auto-fluorescence intensity of the surface without sample) was measured first. The background measured immediately before the sample measurement was always subtracted afterwards from the sample measurement.

The calibration correlates fluorescence intensity of curcumin of the o/w emulsions to oil fraction per surface area of tongue. For this purpose we used pigs tongues at room (21 ± 0.5°C) and body temperature (37.5 ± 0.5°C) as surfaces. For each measurement a fresh piece of tongue was used. Five o/w emulsions at room temperature were spread on the pigs' tongue surface to achieve the target oil fraction (**Table 2.2**).

Oil fraction was defined as:

$$Oil\ Fraction\ (mg/cm^2) = \frac{m_{oil}}{A_{surface\ tongue}}$$

with $m_{oil} = V_{emulsion}[oil]_{emulsion}$

Where m_{oil} is the mass of oil on the tongue, $V_{emulsion}$ is the volume of the emulsion spread on the tongue, $[oil]_{emulsion}$ is the concentration of oil in the emulsion and $A_{surface\ tongue}$ is the surface area of the pigs tongue where the sample is dispersed. The calibrations were made for targeted oil fractions between 0.01 mg/cm² and 1.8 mg/cm² (9 points) at room temperature (21 ± 0.5°C) and body temperature (37.5 ± 0.5°C). All measurements were performed in triplicate.

Table 2.2. Fraction of oil (mg/cm²) used for the calibrations at room (21°C) and body temperature (37.5°C). Each fraction of oil was obtained by spreading the specified volume of o/w emulsion ($V_{\_emulsion}$) with the specified concentration ($[oil]_{emulsion}$) on pigs tongues with a constant surface area of 4 cm².

Oil fraction (mg/cm²)	$V_{emulsion}$ (µL)	[Oil] emulsion (% w/w)
0.01	4	1
0.02	8	
0.15	12	5
0.20	16	
0.50	20	10
0.60	24	
1.05	28	15
1.20	32	
1.80	36	20

2.5.1 *In vivo* Fluorescence Measurements

Twenty untrained subjects (6 men and 14 women, mean age of 24 ± 1.7 years) tested the five o/w emulsions in triplicate. A randomized complete block design (randomization of five products per session) was used. Each sample was served in

plastic cups in doses of 20 ml. The samples were coded with randomized three-digit numbers and served at room temperature. The test was performed in a controlled temperature and ventilated room. All subjects signed a written consent form and were given a financial compensation. The materials and methods used did not require medical ethical approval under Dutch regulations.

Determination of Oil Fraction on Tongue

Each subject was instructed to ingest 20 ml of the o/w emulsion and process the sample in the mouth for 30s while moving the sample around freely before spitting it out. This procedure was followed to create a maximum oil deposition on the tongue rather than to mimic natural drinking behavior. After expectoration fluorescence measurements were made on the front and back of the anterior tongue at 0, 15, 30, 45, 60, 90, 120 and 180s after expectoration. While waiting for the next measurements, subjects were free to swallow saliva and instructed to not speak or drink water. After 180s the subjects were given a recuperation time of approximately five minutes to cleanse their mouth with crackers, tongue scraper, warm (40°C) and room temperature water in order to remove the residue of the oral coating. Before each sample was presented to the subject, a background fluorescence measurement (auto-fluorescence of the tongue) was made and subtracted from the subsequent sample measurement.

Sensory Perception of Oral Coatings

During the fluorescence measurement the subjects were asked to rate the intensity of three sensory attributes: "roughness", "fatty film" and "flavor intensity". These attributes were chosen as they are descriptors that have been generated previously by QDA panels to describe after-feel sensations of o/w emulsions (van Aken *et al.*, 2011). In our study the three attributes were evaluated by the untrained subjects after the stimuli were expectorated. The definition of descriptors and the evaluation protocol are shown in **Table 2.3**. The evaluation was done using a continuous 100 mm VAS line scale anchored with little – very

5% from each end. The intensity of the attributes was rated 0, 30, 60, 90, 120, 180s after expectoration immediately after the fluorescence measurements.

Table 2.3. List of sensory attributes with attribute definitions and evaluation protocol.

Sensory attribute	Definition	Evaluation protocol
Roughness	Roughness sensed on teeth, palate and tongue.	After spitting out the sample: analysis between tongue and palate by sliding, degree of roughness of surface.
Fatty Film	Sensation of feeling a layer of oil covering the mouth.	After spitting out the sample: slide the tongue on the palate and lips and the lips on one another.
Flavor Intensity	The overall flavor intensity of aroma and taste.	After spitting out the sample: the flavor intensity perceived.

Data Analysis

Descriptive statistics was used to obtain the mean and Standard Error (SE). A preliminary test for equal variances and normality was performed. Since these assumptions were not met for all the fluorescence measurements, the data was normalized with a ln(x+1) transformation. The effect of the position of the probe (within subject factor; front back), sample (within subject factor; 1, 5, 10, 15, 20% w/w), time (within subject factor; 0, 15, 30, 45, 60, 90, 120, 180s) and the interactions on the oil fraction of the coating was tested by repeated-measures ANOVA. For the sensory data a repeated-measures ANOVA was used to investigate the effect of samples (within subject factor: 1, 5, 10, 15, 20% w/w) and time (within subject factor: 0, 30, 60, 90, 120, 180s) and the interactions on "roughness", "fatty film" and "flavor intensity" scores. These analyses were performed in SPSS® Statistics version 19. A significance level of $p<0.05$ was chosen.

3. Results

3.1 Stability of o/w emulsions under Mimicked in Mouth Conditions

The stability of the 10% (w/w) o/w emulsion on a pig's tongue was analyzed using CLSM (**figure 2.1**). First, a layer of human saliva was added to the pig's tongue in the absence of the 10% (w/w) o/w emulsion (**figure 2.1 A**). Secondly, the 10% (w/w) o/w emulsion was added to the pig's tongue previously covered with human saliva (**figure 2.1 B**). Thirdly, to mimic the oral processing behavior during drinking (*i.e.* rubbing of the tongue against the palate), the piece of pig's tongue covered with a layer of human saliva and 10% (w/w) o/w emulsion was rubbed against a second pig's tongue (**figure 2.1 C**). The papillae are represented in green, the oil droplets in red. The microstructure of the 10% (w/w) o/w emulsion did not change considerably under the mimicked in-mouth conditions by mechanical stress through rubbing. A qualitative comparison between **figure 2.1 B** and **figure 2.1 C** reveals no noticeable change in the microstructure of the emulsion. **figure 2.1 B** and **figure 2.1 C** both show individual oil droplets adhering to the tongue papillae. No coalescence of oil droplets after application of mechanical stress is observed.

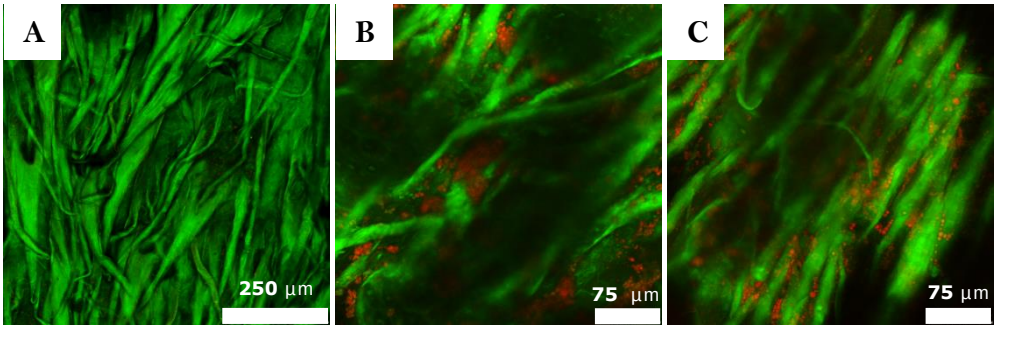

Figure 2.1. Confocal Laser Scanning Microscopy images. (A) Pig's tongue with layer of human saliva, (B) pig's tongue with layer of human saliva and o/w emulsion (10% w/w), (C) pig's tongue with layer of human saliva and o/w emulsion (10% w/w) after being rubbed with another piece of pig's tongue. The same pig's tongue was used to collect the three images. Papillae are represented in green, oil droplets in red.

3.2 Calibration lines for *in vivo* Fluorescence Measurements

Calibration lines were made using pigs tongues as surfaces at room temperature (21 ± 0.5°C) and body temperature (37.5 ± 0.5°C) to mimic in-mouth conditions (**figure 2.2**). Five o/w emulsions were used to achieve the target oil fractions for the calibration lines (**table 2.2**). The five o/w emulsions were the same as the ones used for the *in vivo* fluorescence measurements. The data obtained from both calibrations were fitted with a linear regression to correlate the relative fluorescence units (RFU) with the oil fraction per surface area of tongue ($[oil]_{fraction}$). At room temperature (21 ± 0.5°C), RFU = 0.94 x $[oil]_{fraction}$ (R^2=0.96) was obtained and at body temperature (37.5 ± 0.5°C) RFU = 0.45 x $[oil]_{fraction}$ (R^2=0.94). Both linear regressions passed through the origin. The increase of temperature by 16.5°C decreased the fluorescence intensity by 45%. In the following, the calibration line with the pig's tongue at body temperature was used to calculate the oil fraction deposited on the tongue since that calibration resembles the in mouth conditions closer.

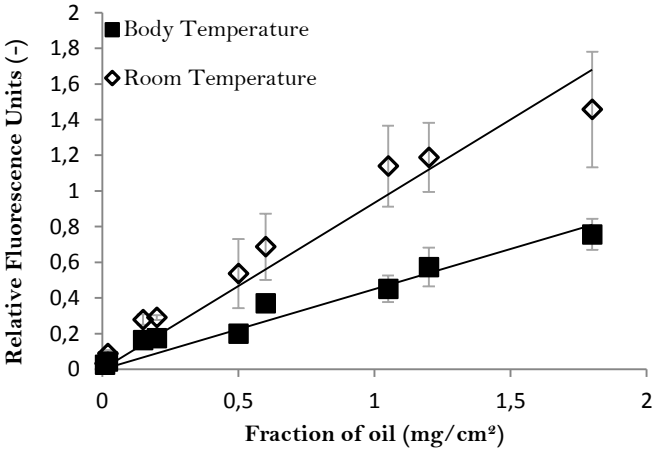

Figure 2.2. Calibration lines. Relating the Relative Fluorescence Units (RFU) to the fraction of oil on tongue surface ($[Oil]_{fraction}$). Error bars represent the standard deviation.

3.3 Influence of Oil Content of o/w emulsions on Spatial Variation of Oral Coatings

Figure 2.3 A and **2.3 B** show the decay of the oil fraction deposited on the tongue after expectoration over time. The effect of probe position, sample, time and their interactions on the oil fraction were statistically analyzed. Mauchly´s test indicated that the assumption of sphericity had been violated for the main effect of oil content of emulsions and the interactions. Therefore, the degrees of freedom were corrected using Greenhouse-Geisser estimates for sphericity. All effects are significant at $p<0.001$. There was a significant main effect of probe position [$F(1, 59)=166.5$], sample [$F(3.7, 215.9)=52.5$], time [$F(7, 413)=620.4$] and probe position x sample x time [$F(15.3, 903.6)= 2.07$, $p=0.009$]. The oil fraction deposited on the front of the anterior tongue was significantly lower than on the back of the anterior tongue. The oil deposition on the front decreased in average by 42% after 15s and by 67% after 45s after expectoration. After 90s no significant difference of oil fraction at the front of the anterior tongue was found between emulsions (**figure 2.3 A**). At the back of the anterior tongue the oil fraction decreased in average by 34% after 15s and by 60% after 45s after expectoration (**figure 2.3 B**). After 120s there was no significant difference between emulsions. We conclude that the oil clearance was faster at the front of the anterior tongue compared to the back of the anterior tongue. The oil fraction on the front and back of the anterior tongue differed by a factor of 1.8 at 0s, 2.2 at 30s and 2.4 at 180s after expectoration.

The oil fraction deposited on the front and back of the anterior tongue surface immediately after expectoration of the stimulus (0s) increased linearly with oil content of the o/w emulsion (**figure 2.3 C**).

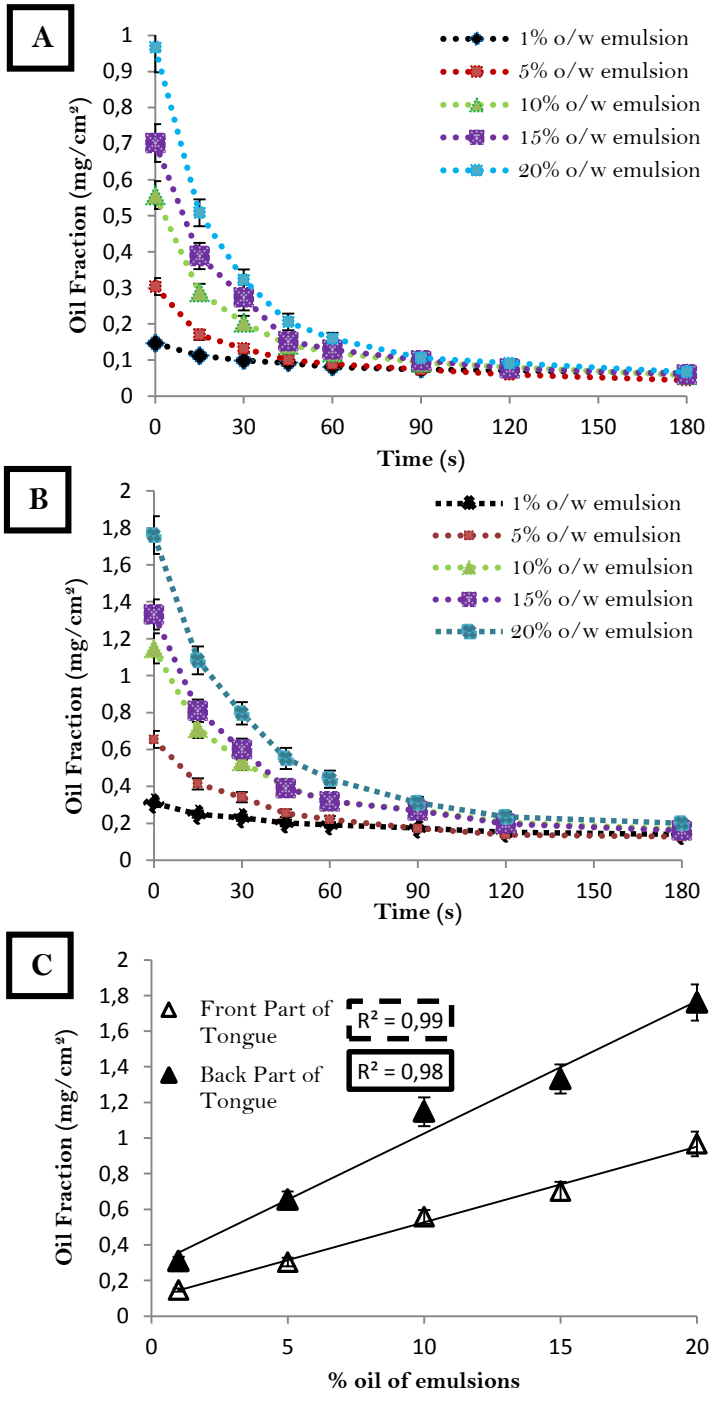

Figure 2.3. Oil fraction deposited on front (A) and back (B) of the anterior tongue over time for 5 o/w emulsions differing in oil concentration. Each point represents the average

of n= 20 subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent the standard error **Figure 2.3** (**C**) shows the oil fraction deposited at the front and back of the anterior tongue immediately after expectoration (0s) as a function of emulsion oil concentration.

3.4 After-feel Perception of Oral Coatings

Figures 2.4A and **2.4B** show the averaged intensities of the attributes "fatty film" and "flavor intensity" of the five o/w emulsions over time. The effect of sample, time and sample x time on the attributes "fatty film", "flavor intensity" and "roughness" (data not shown) was tested. All effects were tested for sphericity with the Mauchly´s test. The effects which violated the sphericity assumption were corrected by Greenhouse-Geisser estimates.

For the attribute "roughness" all effects were not significant (sample $p=0.11$, time $p=0.063$ and sample x time $p=0.175$).

For the attribute "flavor intensity" the effects of sample [$F(3.2, 189.1)=25.3$], time [$F(1.8, 108.6)=94.0$] and the interaction sample x time [$F(9.5, 562.3)=6.2$] were significant at $p<0.001$.

The attribute "fatty film" was significantly influenced by the same effects: sample [$F(4, 236)=15.2$, $p<0.001$], time [$F(1.6, 91.6)=117.5$, $p<0.001$] and the interaction sample x time [$F(9.3, 548.3)=2.3$]. At time 0s both attributes were perceived as more intense with increasing oil content of o/w emulsions (**figure 2.4 A** and **2.4 B)**. For both attributes, the 1% (w/w) o/w emulsion was perceived as significantly different from all other samples up to 120s ($p<0.001$). At time point 180s all samples were perceived equally intense. The attributes "fatty film" and "flavor intensity" were linearly correlated for all samples ($R^2> 0.98$, regression data not shown).

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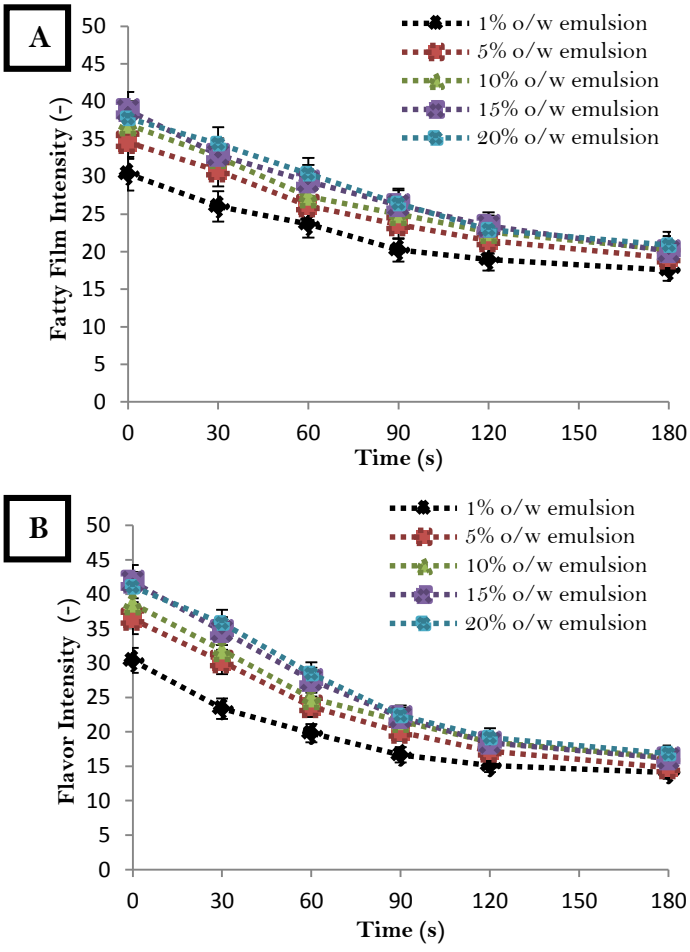

Figure 2.4. Intensity of the attributes Fatty film (A) and Flavor Intensity (B) of five o/w emulsions over time. Each point represents the average

3.5 Psychophysical Relationships Between Oil Coatings formed on the Tongue and After-feel Perception

Figure 2.5 A and 2.5 B show the relationships between the after-feel perception of attributes "fatty film" and "flavor intensity" with oil fraction deposited on the tongue. Each data point represents a different sample at a different time point after expectoration of the stimulus. The perceived intensity of a stimulus can be related to the physical intensity of the stimulus by a logarithmic relationship

known as Weber-Fechner's law: $P = k \log I$ where P is the perceived intensity of the stimulus, I the physical intensity of the stimulus and k a constant. The psychophysical relationships between the attributes "flavor intensity" and "fatty film" and the oil fraction deposited on the tongue after expectoration of the stimulus were fitted using Weber-Fechner's law to determine the psychophysical functions for after-feel perception of the o/w emulsions (**figure 2.5 A and 2.5 B**). Weber-Fechner's law described the relationships between after-feel perception of the two attributes and oil fraction ($R^2 \geq 0.90$).

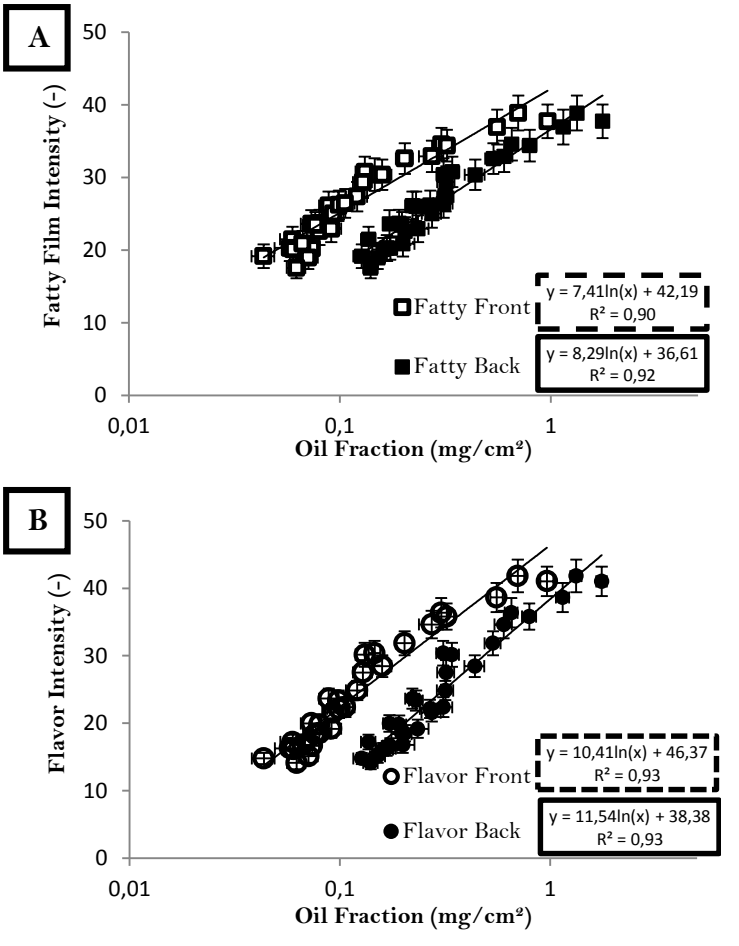

Figure 2.5. Fatty film intensity (A) and Flavor Intensity (B) as a function of oil deposition of different emulsions at the front and back of the anterior tongue at 0s, 30s, 60s and 90s. Each

point represents the average of n= 20 subjects and 3 replicates. Lines represent the linear regression of all points. Error bars represent the standard error.

4. Discussion

4.1 Stability of o/w emulsions under Mimicked in Mouth Conditions

The stability of the 10% (w/w) o/w emulsion was qualitatively determined under mimicked in-mouth conditions using CSLM. The oil droplets remained stable and did not reveal coalescence after application of mechanical stress through rubbing the pigs tongue covered with the emulsion against another pigs tongue. Dresselhuis *et al* (2008b) reported that the occurrence of coalescence in mouth depends on the characteristics of the emulsion (droplet size, interfacial layer, type of emulsifier and type of fat), the shear applied and the characteristics of the oral mucosa. The coalescence in mouth was shown to be more probable when the emulsions were less stable *i.e.* stabilized by a low concentration of emulsifier (Dresselhuis *et al*., 2008b). The concentration of emulsifier used in the preparation of all our emulsions (3.2% w/w Na-caseinate) was in excess to cover the oil/water interface. Therefore, as expected the oral coating formed by the emulsion consists of individual oil droplets embedded in a mixture of water and saliva adhering to the tongue papillae and does not consist of a homogeneous layer of coalesced oil. Hence, thickness should not be used as the physical parameter to describe and quantify oral coatings formed by (stable) emulsions. Oil fraction is a more suitable physical parameter to describe the mass of oil that adheres to the surface of the tongue.

4.2 Calibration lines for *in vivo* Fluorescence Measurements

The first aim of this study was to develop a new calibration method for *in vivo* fluorescence measurements which represents closer the *in vivo* conditions occurring in mouth. Pigs tongues were used as contact surface for the calibration as they are known to be a good model for human tongues (Dresselhuis *et al*., 2008a, Kulla-Mikkonen *et al*., 1987). The calibration of the fluorescence method

was made with o/w emulsions. To test the effect of temperature on fluorescence, calibrations were performed with pigs tongues at body temperature and room temperature. We observed a large effect of temperature on the fluorescence intensity of curcumin in the emulsions on the tongue surface. This demonstrates clearly the importance of performing the calibration at temperatures close to those occurring during the *in vivo* measurements in the oral cavity. The experimental conditions of the calibration should resemble the in mouth conditions during the *in vivo* measurements as much as possible.

4.3 Influence of Oil Content of o/w emulsions on Spatial Variation of Oral Coatings

The second aim of this study was to apply the *in vivo* fluorescence method with the new calibration using o/w emulsions varying in oil content. The *in vivo* fluorescence measurements showed more oil deposition on the back of the anterior tongue than on the front of the anterior tongue (**figure 2.3 A** and **2.3 B**). This spatial variation of the oil fraction increased over time. An explanation for this finding may lay in the morphology of the tongue. Fungiform papillae which are the smallest papillae are present on the front of the anterior tongue. The papillae circumvallate are located on the back of the anterior tongue and secret cleaning liquid (Bear *et al.*, 2001). The most important papillae for the formation of the oral coating might be the filiform papillae which are more predominant at the back of the tongue. These papillae contribute considerably to the roughness of the tongue surface. The differences between the papillae result in a smoother surface at the front of the anterior tongue compared with the back (Kawasaki *et al.*, 2012). The difference in the surface morphology might partly explain the observed differences in spatial variation of oral oil deposits since oil droplets are likely to be entrapped between the papilla. The movement of the tongue might also influence the spatial variation of the oil deposit. The participants were allowed to swallow freely during the experiments. When swallowing the front of

the tongue moves against the teeth and palate (ChiFishman *et al.*, 1996, Pouderoux *et al.*, 1995). This movement results in friction which might degrade the oil coating more on the front of the anterior tongue compared with the back. This effect might also explain the increase in spatial variation over time. The oil fraction deposited on the tongue surface immediately after expectoration (0s) increased linearly with increasing oil content of the o/w emulsion (**figure 2.3 C**). Dresselhuis *et al.*, (2008b) studied the effect of stable and unstable emulsions on the oil retention in mouth. It was concluded that increasing the oil content of the emulsions resulted in larger amounts of oil deposition on the tongue (Dresselhuis *et al.*, 2008b). In contrast, results by Pivk *et al.*, (2008b) showed that deposition of MCT oils in mouth followed a logarithmic increase with increasing volumes of oil (Pivk *et al.*, 2008b). A reason for the difference between these results may lay in the level of saturation of the oil coverage of the surface of the tongue. Complete coverage of the tongue surface by oil (saturation) is more likely to be reached by oils than by emulsions that contain considerably less oil and the oil droplets can be stable against coalescence.

4.4 After-feel Perception of Oral Coatings

The decrease of oil fraction on the tongue with time lead to a decrease of "fatty film" and "flavor intensity" with time (**figure 2.4 A** and **2.4 B**). The oral coating might lead to a prolonged release of odor compounds into the nasal cavity and to a higher lubrication in mouth. Hence, more coating might result in a higher concentration of odors and thus increase flavor perception (Prinz *et al.*, 2006). The increase of after-feel intensity at t=0s with increasing emulsion oil content was not linear probably since the flavor release also does not depend linearly on oil content (Dresselhuis *et al.*, 2008b). It should be noted that no flavor substances were added to the emulsion to examine flavor release over time. The flavor of the o/w emulsions comes from the inherent flavor of the sunflower oil. The attribute "flavor intensity" was used as an indirect measurement of the presence of an oral coating. The attribute "roughness" showed no significant differences for any of

the effects tested. This might have been due to a low consensus for the attribute "roughness" between the untrained subjects. The definition of "roughness" might have been unfamiliar or unclear to the untrained subjects leading to misinterpretations or confusion. This effect would probably have been suppressed when a trained panel would have been used.

4.5 Psychophysical Relationships between Oil Coatings formed on the Tongue and After-feel Perception

The third aim of the study was to establish psychophysical relationships between oil coatings formed on the tongue and after-feel perception. For both attributes "fatty film" and "flavor intensity" a semi-logarithmic relation with the oil fraction of the coating was found (**figure 2.5 A** and **2.5 B**). The relationship between after-feel perception of the attributes "fatty film" and "flavor intensity" and oil fraction deposited on the tongue can hence be described by Weber-Fechner´s law. The *k* values of Weber-Fechner's law are an indication for the sensitivity of the sensory system towards a specific physical stimulus. In our study we cannot determine a meaningful *k* value from our data since we used a direct intensity scaling method to quantify after-feel perception. In order to determine the *k* value of Weber-Fechner's law usually indirect methods such as 2AFC's and/or method of constant stimuli and/or magnitude estimation methods are used (Schutz *et al.*, 1957, Withers *et al.*, 2013, Hoppert *et al.*, 2012, Orellana-Escobedo *et al.*, 2012). The indirect methods are known to work well to quantify k values for taste and smell perception. Our study demonstrates that after-feel perception of o/w emulsion is dynamic and intensity of attributes depends strongly on time after expectoration. Using indirect sensory methods to determine after-feel perception while accounting for perceptual changes of after-feel in time will be challenging to perform. Since we cannot determine a meaningful *k* value from our data, we cannot compare it with *k* values reported in literature for other sensory systems such as taste and smell.

In conclusion, we developed a new calibration method for *in vivo* fluorescence measurements and applied the methodology to investigate the psychophysical relationships between oil fraction deposited on the tongue after expectoration of o/w emulsions and after-feel perception. We recommend a new calibration method using pigs tongue surfaces at body temperature for the calibration procedure to resemble in mouth conditions. We conclude that the after-feel perception of oral coatings follows Weber-Fechner's law. This study demonstrates the potential of *in vivo* fluorescence measurements for studying food behavior in mouth. We suggest to continue the studies focusing on the effect of beverage composition on the formation and clearance of oral coatings for improved design of low-fat products.

Abbreviations Used:

2-AFC, two alternative forced choice; ANOVA, analysis of variance; ATR IR, attenuated total reflection infra-red; CLSM, confocal laser scanning microscopy; MCT, medium-chain triglycerides; o/w, oil in water; RFU, Relative Fluorescence Units; VAS, visual analogue scale.

Properties of Oil/Water Emulsions Affecting the Deposition, Clearance and After-feel Sensory Perception of Oral Coatings

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Abstract

The aims of this study were to investigate the influence of (i) protein type, (ii) protein content and (iii) viscosity of o/w emulsions on the deposition and clearance of oral oil coatings and after-feel perception. Oil fraction (moil/cm2tongue) and after-feel perception differed considerably between emulsions which do not flocculate under in mouth conditions (Na-caseinate) and emulsions which flocculate under in mouth conditions (lysozyme). The irreversible flocculation of lysozyme stabilized emulsions caused slower oil clearance from the tongue surface compared to emulsions stabilized with Na-caseinate. Protein content had a negative relation with oil fraction for lysozyme stabilized emulsions and no relation for Na-caseinate stabilized emulsions, immediately after expectoration. Viscosity differences did not affect oil fraction, although the presence of thickener decreased deposition of oil on tongue. We conclude that after-feel perception of o/w emulsions is complex and depends on the deposited oil fraction, the behavior of proteins in mouth and thickeners.

Keywords: oral coating, *in vivo* fluorescence measurements, emulsion, Na-caseinate, lysozyme, xanthan, after-feel perception

1. Introduction

Oral coatings are defined as residues of foods and beverages that are retained in the oral cavity after swallowing. Oral coatings are known to influence taste, texture and after-feel perception of foods (de Wijk *et al.*, 2009, Ahn *et al.*, 2002, Lynch *et al.*, 1993, Madrigal-Galan *et al.*, 2006, Camacho *et al.*, 2014). The formation and clearance of oral coatings on the tongue surface is hypothesized to depend on several factors such as the composition of the food (type and amount of fat, protein and polysaccharide), the physical-chemical properties of the food (rheological properties, pH) and the morphology of the tongue which can vary between individuals (de Wijk *et al.*, 2009, Ahn *et al.*, 2002, Lynch *et al.*, 1993, Madrigal-Galan *et al.*, 2006, Camacho *et al.*, 2014, Dresselhuis *et al.*, 2008b, Ranc *et al.*, 2006, Vingerhoeds *et al.*, 2009, Pivk *et al.*, 2008b)

Several studies have reported relations between oral coatings and after-feel perception (de Wijk *et al.*, 2009, Camacho *et al.*, 2014, Ranc *et al.*, 2006, Vingerhoeds *et al.*, 2009, Pivk *et al.*, 2008b). After-feel perception of oil/water (o/w) emulsions was found to follow a semi-logarithmic relationship to the oil fraction deposited on the tongue surface (m_{oil}/cm^2_{tongue}) after expectoration of the emulsion (Camacho *et al.*, 2014). Perceived fattiness of custards was found to increase with increasing amount of oral coating (de Wijk *et al.*, 2009). Likewise, perception of creamy and coating after-feel and fatty and slippery mouth-feel increased with increasing oil content of o/w emulsions (van Aken *et al.*, 2011). It was hypothesized that the retention of emulsion droplets onto the oral mucous layer gives rise to lubrication in the oral cavity leading to an enhancement of perception of fat related attributes. When gum arabic was added as a thickener to low oil content o/w emulsions, the perception of coating after-feel, fatty and slippery mouth-feel increased (van Aken *et al.*, 2011). This suggests that thickeners contribute to sensory perception of fat related attributes in o/w emulsions. It was hypothesized that a thickener can form a layer similar to oil on oral surfaces or imitate an oil layer due to increased viscosity in mouth

(Vingerhoeds *et al.*, 2009). Conversely, addition of guar gum to o/w emulsions resulted in a slightly reduced oil retention on the tongue surface (Vingerhoeds *et al.*, 2009). No differences were reported in oil retention on the tongue between dressings differing in type of thickeners (starch, xanthan and a mixture of the two) (de Jongh *et al.*, 2007).

In addition to viscosity, the behavior of o/w emulsions in mouth may also affect coating deposition and after-feel perception. The behavior of o/w emulsions in mouth depends on the protein used to stabilize the o/w emulsions (Silletti *et al.*, 2010, 2007). Flocculation of o/w emulsions can occur when emulsions are mixed with saliva (Siletti *et al.*, 2010, 2007). Whether the flocculation is reversible or not depends on the surface charge of the emulsion droplet, pH, salts and composition of salivary biopolymers. Saliva is charged negatively at neutral pH (Silletti *et al.*, 2007). Emulsion droplets stabilized with highly negative charged proteins do not flocculate with salivary biopolymers, while weakly negative charged to neutral surface charged emulsion droplets experience reversible flocculation (Silletti *et al.*, 2007). Positively surface charged emulsion droplets flocculate irreversibly with negative biopolymers in saliva due to bridging interactions (Silletti *et al.*, 2007). Previous work focused on the in mouth behavior of lysozyme, a protein with a net positive charge at neutral pH. It was found that flocculation due to electrostatic interactions occurred when o/w emulsions stabilized with lysozyme were mixed with saliva (Silletti *et al.*, 2007).

In addition to protein charge, also protein content can play a role on the coating deposition and after-feel perception. The effect of protein-poor (or unstable) against protein-rich (or stable) o/w emulsions on the adhesion and spreading of fat on the tongue was previously reported (Dresselhuis *et al.*, 2008b). It was found that o/w emulsions stabilized with little protein had a stronger adhesion on the tongue (*i.e.*, more stable against rinsing with saliva) compared to o/w emulsions stabilized with high concentration of protein. It was hypothesized that this was

due to difference in stability of the interfacial layer of the emulsion against rupture, and thus creating coalescence of the oil droplets (Ranc *et al.*, 2006)

Although the mentioned studies have indicated relations between o/w emulsion properties, such as viscosity, emulsifier type and content, and oral coatings, the mechanisms on how different macronutrients influence the deposition and clearance of oral oil coatings and how they relate to after-feel perception are still not understood. The aims of this study are to investigate the influence of (i) protein type, (ii) protein content and (iii) viscosity of o/w emulsions on the clearance of oral oil coatings and after-feel sensory perception. The deposition and clearance of oral oil coatings from the tongue surface was determined using *in vivo* fluorescence measurements. The after-feel perception was determined using progressive profiling. To study the influence of protein emulsifier type on oil coatings, o/w emulsions stabilized with proteins differing in flocculation behavior when mixed with saliva were prepared. Lysozyme which forms irreversible agglomerates upon mixing with saliva and Na-caseinate which is hypothesized to form no agglomerates upon mixing with saliva were used (Silletti *et al.*, 2007). To study the influence of protein content on clearance and after-feel sensory perception of oral oil coatings, three concentrations of protein were used (all in excess to stabilize the water/oil interface). We hypothesize that excess protein has a blending effect with saliva resulting in faster clearance of oil deposits from the tongue surface. To study the influence of viscosity of o/w emulsions on the clearance and after-feel sensory perception of oral oil coatings, three o/w emulsions stabilized with Na-caseinate were thickened with varying concentrations of xanthan gum. Emulsions stabilized with Na-caseinate were chosen to study the influence of viscosity, independently from other factors, to rule out possible effects of emulsifier-saliva biopolymers. Xanthan gum was chosen as it is commonly used as a thickener in various food liquid foods.

2. Materials and Methods

2.1 Materials

Sunflower oil (Perfekt, purchased from local retailer), Na-caseinate (Excellion sodium caseinate S, DMV International, The Netherlands, typical protein content: 91%), lysozyme hydrochloride (The Protein Company, Belgium, protein content: > 99%), sucrose (AH basic, purchased from local retailer), xanthan gum (Keltrol Advanced Performance, CP Kelco, Denmark), curcumin (7% w/w curcumin dissolved in propylene glycol and polysorbate; L-WS; Sensient, The Netherlands), NaOH (Merck, Germany) and tap water were used. All ingredients were food grade. Pig's tongues were obtained from the VION Food Group (The Netherlands).

2.2 Preparation and Characterization of o/w emulsions

Table 3.1 shows the composition of all the o/w emulsions. All o/w emulsions were prepared in a food-grade environment. The oil phase of the o/w emulsions consisted of sunflower oil (10% (w/w)). The aqueous phase of the o/w emulsions consisted of protein solution (concentrations see **table 3.1**) with 8% (w/w) sucrose. The aqueous phase was prepared by first dissolving sucrose in tap water, and later dissolving either Na-caseinate or lysozyme, at room temperature. The aqueous phase with dissolved lysozyme was brought to a pH of 6.9 by addition of 1M NaOH. Protein concentration in the aqueous phase varied (0.2%, 3.0% and 5.8% (w/w)). The 0.2% (w/w) protein concentration was chosen, as the lowest concentration, as it allows to prepare stable emulsions with low concentrations of "free-protein" in the aqueous phase, *i.e.*, protein that is not adsorbed into the oil droplets surface. The 3.0% (w/w) protein concentration was chosen, as the middle concentration, as it is a protein concentration common in dairy drinks. The 5.8% (w/w) protein concentration was chosen, as the highest concentration, as it creates emulsions with high concentration of "free-protein" in the aqueous phase

and has an equal difference of protein concentration between the emulsion with 3.0%, as the emulsion with 0.2%.

The aqueous phase was then pre-homogenized with the oil phase (sunflower oil) using an Ultra Turrax (IKA® RW 20 Digital) at 4000 min^{-1} for 3 minutes, and homogenized with a two-stage homogenizer (Niro-Soavi S.p.A. NS1001L2K) at pressures between 250 and 350 bar. All o/w emulsions had a neutral pH. The o/w emulsions thickened with xanthan gum were prepared from an o/w stock emulsion and mixed at a ratio of 1:1 with pre-prepared aqueous xanthan gum solutions. Xanthan gum solutions were prepared by heating water to 70°C and adding the xanthan gum under agitation. The xanthan gum thickened o/w emulsions contained 10% (w/w) oil, 3% (w/w) Na-caseinate and 8% (w/w) sucrose in the aqueous phase and varying concentrations of xanthan gum (see **table 3.1**).

Emulsions were stored after preparation in a refrigerator at 4°C and used for further studies no later than three days after preparation. Curcumin was added to the emulsions as a hydrophobic fluorescent dye prior to the *in vivo* measurements. One µL of 7% curcumin per gram of o/w emulsions was added under magnetic stirring immediately before testing.

Particle size distribution was determined by light scattering (Malvern Instruments, Goffin Meyvis) using a refractive index for sunflower oil of 1.469. Particle size of the different o/w emulsions was measured for every batch made for the fluorescence measurements. Sample was added to the light scattering measurement device until the obscuration reached 10%. $d_{3,2}$ was obtained as a measure of oil droplet size and averaged over measurements of 3-4 batches.

For every emulsion, the number of oil droplets was estimated by calculating: $Number\ of\ Oil\ Droplets\ (N) = \frac{Volume\ of\ Oil}{\frac{4}{3}\times\pi\times r^3}$ where r is radius of the oil droplet. By knowing the number of oil droplets in each emulsion, the surface area of the emulsion can be estimated by calculating: $Surface\ Area\ of\ Emulsion = N \times 4 \times \pi \times r^2$. Based on previous studies we can estimate the amount of protein needed to cover the surface area of the emulsions (Hunt *et al.*, 1994).

We estimate that for the o/w emulsions with lower protein concentrations (0.2% w/w) about 1 mg/m² (Na-Caseinate) to 1.5 mg/m² (lysozyme) of protein is adsorbed onto the surface of the oil droplets. For the higher protein concentrations used in our study (> 2.25% (w/w)), we estimate that about 3.2 mg/m² of protein (Na-Caseinate and lysozyme) is adsorbed onto the surface of the oil droplets (Hunt *et al.*, 1994). This corresponds to a minimum protein concentration absorbed on the oil droplet interface of 0.05% (w/w) for low and 0.15% (w/w) for high protein concentrations. Therefore, all protein concentrations used in our study to emulsify the o/w emulsions are assumed to be higher than the minimum protein concentration required to cover the oil droplet surface (see **table 3.1**). All o/w emulsions used contained an excess of protein as emulsifier.

Flow curves of all o/w emulsions were determined in duplicate at 20°C at shear rates ranging from 0.01 to 1000 s^{-1} using a rheometer (Anton Paar GmbH, Physica MCR 301) with a double gap geometry. All samples displayed shear thinning behavior. The viscosities at a shear rate of 63s^{-1} (closest value in the range of the reported in-mouth shear rate (50-100 s^{-1})) were extracted from the flow curves and are reported in **table 3.1**.

Table 3.1. Overview of o/w emulsions. All o/w emulsions contained 10 % (w/w) oil and 8% (w/w) sucrose. O/w emulsions differed in type and concentration of protein used as emulsifier and xanthan gum concentration. $d_{3,2}$- droplet size (averages of measurements of 3-4 batches) and viscosity at a shear rate of $63s^{-1}$ are shown.

o/w emulsion	Protein (% w/w)	Xanthan gum (% w/w)	$d_{3,2}$ ± stdev (µm)	η ± stdev at $63s^{-1}$ (mPas)
Na-caseinate	0.2	0	2.04 ± 0.20	1.7 ± 0.01
	3.0	0	1.45 ± 0.08	5.0 ± 0.05
	5.8	0	1.46 ± 0.05	9.0 ± 0.07
Lysozyme	0.2	0	1.16 ± 0.24	2.2 ± 0.05
	3.0	0	1.21 ± 0.28	2.3 ± 0.05
	5.8	0	1.35 ± 0.19	3.5 ± 0.13
Na-caseinate	3.0	0.05	1.28 ± 0.10	13.8 ± 0.15
	3.0	0.20		49.9 ± 1.50
	3.0	0.50		143 ± 7.28

Light microscopy (Carl Zeiss, Axioskop 2 Plus) was used to characterize the structure of the o/w emulsions before and after mixing with human saliva at a magnification of 40x. Saliva was collected according to reported procedure (Silletti *et al.*, 2007). Unstimulated saliva was collected for 30 minutes in the same morning as the experiments from one healthy subject. First the subject rinsed her mouth with water and then collected the saliva with closed lips and expectorated into ice chilled beakers (Silletti *et al.*, 2007). Mixtures between o/w emulsions and saliva were prepared at room temperature at a ratio of 1:1 as in previous reports (Cook *et al.*, 2003, Vingerhoeds *et al.*, 2005).

2.3 *In vivo* Fluorescence Measurements to Determine Oil Fraction Deposited on Tongue Surface

Fluorescence Measurements and Calibration Curves

The method used in the present study has been previously described in detail.[5] Fluorescence measurements were made with a single point measurement (Fluorolog Instruments SA Inc, Jobin Yvon Spex) at an excitation wavelength of 440 nm, an emission wavelength of 495 nm with a slit width of 0.95 mm and a measurement time of 0.1s. A fluorescence remote read fiber optic probe was used to measure on tongue surfaces (*in vivo* for determination of oil fraction, and *ex vivo* for calibration curves). A plastic ring (diameter 16.9 mm, height 5 mm) was attached to the end of the probe to ensure that the distance between the probe and the tongue surface remained constant. The probe was put perpendicularly to the tongue surface and with slight pressure to avoid deformation of the papillae and the coating. For all measurements the background (fluorescence intensity of the tongue surface without o/w emulsion) was measured first. The background measured immediately before the measurement of the o/w emulsion was subtracted afterwards from the o/w emulsion measurement.

To convert the fluorescence intensity measured on the subjects tongue surface to the oil fraction deposited on the tongue surface, *i.e.* mass of oil per area of tongue (mg/cm^2), calibration lines were made following the procedure described previously (Camacho *et al.*, 2014). Briefly, calibrations were made with pieces of the middle part of the pig's anterior tongue at 37.5^{o}C. O/w emulsions at room temperature were spread on the surface of the pig's tongue (2x2 cm) and fluorescence intensity was measured. For each measurement a fresh piece of tongue was used. As the protein type and content affects the fluorescence intensity of curcumin, calibrations (fluorescent intensities *vs.* oil fraction on tongue surface) were made for each of the nine o/w emulsions. Calibration lines consisted of six data points, for targeted oil fractions of 0.10 mg/cm^2 ($V_{emulsion}$=4μL), 0.20 mg/cm^2 ($V_{emulsion}$=8μL), 0.50 mg/cm^2 ($V_{emulsion}$=20μL), 0.63 mg/cm^2 ($V_{emulsion}$=25μL), 0.88

mg/cm² ($V_{emulsion}$=35µL), and 1.0 mg/cm² ($V_{emulsion}$=40µL). All calibration measurements were performed in triplicate and are shown in the appendix.

Presentation of o/w emulsions

O/w emulsions were presented to the subjects at room temperature in three blocks. Each block consisted of two sessions of about one hour during which each o/w emulsion was presented in triplicate. The o/w emulsions were presented in randomized order over subjects per block. During the first block the o/w emulsions stabilized with 0.2, 3.0 and 5.8% (w/w) Na-caseinate were assessed. During the second block the o/w emulsions stabilized with 0.2, 3.0 and 5.8% (w/w) lysozyme were assessed. Finally, during the third block the o/w emulsions stabilized with 3% Na-caseinate and thickened with 0.05, 0.2 and 0.5% (w/w) xanthan gum were assessed.

Determination of Oil Fraction Deposited on Tongue Surface

Twenty untrained subjects (7 men, 13 women, mean age 23.4 ± 3.2 years) assessed the nine o/w emulsions in triplicate. Only subjects without tongue piercings and braces participated in the study. Participants gave informed written consent and received a financial compensation for their participation.

Subjects were asked to ingest 20 mL of the o/w emulsions and let it flow freely in the mouth for 30 s. Then, subjects expectorated the o/w emulsions and the fluorescence intensity was measured on the front and back part of the anterior tongue at 0, 15, 30, 45, 60, 90, 120 and 180 s after expectoration. Before presentation of the next o/w emulsion, subjects cleaned their tongue with a tongue scraper and crackers were provided together with warm water (40°C) and room temperature water to rinse the oral cavity. A break of approximately 5 min was made between measurements.

Determination of After-feel Perception of Oral Coatings

During the fluorescence measurements, subjects performed progressive sensory profiling of after-feel attributes fatty film, sweetness and creaminess at 0, 30, 60, 90, 120 and 180 s after expectorating the o/w emulsions. The definition of attributes and evaluation protocol were explained to the subjects (**table 3.2**). The three attributes for the progressive profiling were chosen as they have been shown to relate to the after-feel perception of o/w emulsions after expectoration (van Aken *et al.*,2011; Pivk *et al.*, 2008). A continuous 100 mm Visual Analogue Scale was used anchored with "little" and "very" at a distance of 5 mm from the ends.

Table 3.2. **Overview of after-feel attributes** assessed during progressive profiling at different times points after expectorating (0, 30, 60, 90, 120 and 180 s) of o/w emulsions with definitions and evaluation protocol.

Sensory Attribute	Definition	Evaluation Protocol
Fatty Film	Sensation of feeling a layer of oil covering the mouth	Slide the tongue against the palate and lips and slide the lips against each other
Sweetness	Degree of sweetness that lingers in the mouth	Evaluate the sweetness perceived after expectoration
Creaminess	Degree of creaminess perceived in the mouth	Slide the tongue against the palate and evaluate degree of creaminess

Statistical data analysis

SPSS® Statistics version 19 was used for the statistical analysis. Descriptive statistics were used to obtain the mean and standard error (SE). Outliers ($z>2$) were removed from the data. Fluorescence intensity data was normalized with a square root transformation. The effect of position of probe (within subject factor; front and back), o/w emulsion (within subject factor; 0.2, 3.0, 5.8% lysozyme; 0.2, 3.0, 5.8% Na-caseinate; 0.05, 0.2, 0.5% xanthan), time (within subject factor; 0, 15,

30, 45, 60, 90, 120, 180s after expectoration) and interactions on oil fraction deposited on the tongue were tested by repeated-measures ANOVA. For the sensory data, a repeated-measures ANOVA was used to investigate the effect of o/w emulsion (within subject factor; 0.2, 3.0, 5.8% lysozyme; 0.2, 3.0, 5.8% Na-caseinate; 0.05, 0.2, 0.5% xanthan) and time (within subject factor: 0, 30, 60, 90, 120, 180s after expectoration) and interactions on "fatty film", "sweetness" and "creaminess" intensity. A significance level of $p<0.05$ was chosen.

3. Results

3.1 Influence of Protein Type and Content on Oil Deposition on Tongue

Microstructure of o/w emulsions Stabilized with Na-caseinate and Lysozyme Before and After Mixing with Saliva

Figure 3.1 shows the microstructure of 10% (w/w) o/w emulsions stabilized with different concentrations of Na-caseinate and lysozyme before and after mixing with human saliva. The 0.2% Na-caseinate o/w emulsion showed separate oil droplets (*i.e.* no aggregation) before and after mixing with human saliva. The o/w emulsions stabilized with 3.0 and 5.8% w/w Na-caseinate showed aggregation of the emulsion droplets. The size of the aggregates of the 3.0 and 5.8% w/w Na-caseinate emulsions seemed to decrease slightly upon mixing with saliva. The o/w emulsions stabilized with Na-caseinate show no strong interaction with saliva since no considerable changes in the microstructure of the emulsions are observed comparing the microstructure before and after mixing with saliva. All lysozyme stabilized o/w emulsions (0.2, 3.0 and 5.8% (w/w) lysozyme) show individual, separate oil droplets before mixing with saliva. O/w emulsions stabilized with lysozyme revealed no flocculation at any of the lysozyme concentrations studied here. Upon mixing with saliva, agglomeration of lysozyme stabilized oil droplets was observed for all lysozyme concentrations.

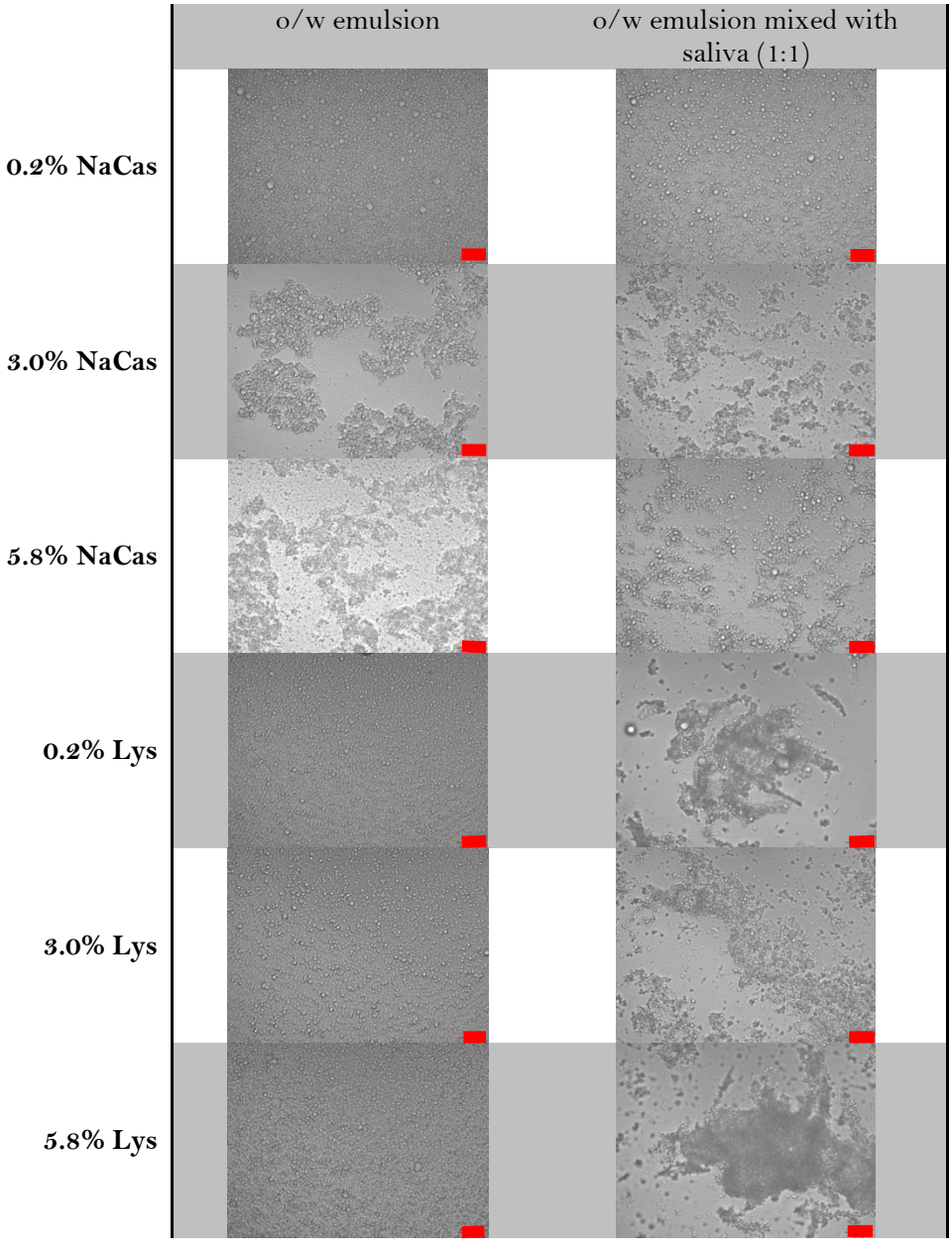

Figure 3.1. Light microscopy pictures. Left column: Images of 10% (w/w) o/w emulsions before mixing with human saliva and (right column) after mixing with human saliva at a ratio of o/w emulsion : saliva of 1:1. Scale bars represent 20μm.

Oil Deposition on Tongue Immediately after Expectoration of o/w emulsions (t=0s)

Figure 3.2 shows the oil fraction deposited on the tongue surface immediately after expectoration (t=0s) of the 10% (w/w) o/w emulsions stabilized with different concentrations of Na-caseinate and lysozyme at the front and back of the anterior tongue. Probe position had a significant main effect on oil fraction deposited on the tongue for emulsions stabilized by Na-caseinate as well as lysozyme (Na-caseinate: $F(1,59)=199$, $p<0.001$; lysozyme: $F(1,59)=260$, $p<0.001$).

The oil fraction on the back part of the tongue was significantly higher than on the front part of the anterior tongue. No significant effect of Na-caseinate concentration on the oil fraction deposited on the tongue was found. With increasing lysozyme concentration the oil fraction deposited on the tongue significantly decreased ($p<0.05$).

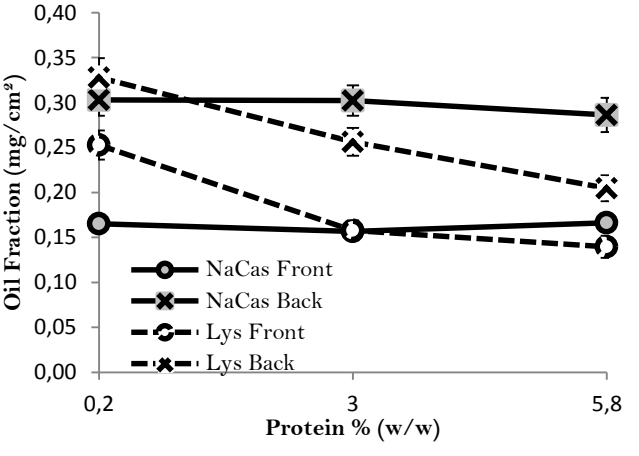

Figure 3.2. Oil fraction deposited on front and back of anterior tongue immediately after expectoration (t=0s) as a function of protein concentration (% w/w) and type Na-caseinate (NaCas) and lysozyme (Lys). Each data point represents the average of n= 20 subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent standard error.

Clearance of Oil Deposition on the Tongue after Expectoration

Time after expectoration had a significant main effect on oil fraction deposited on the tongue for o/w emulsions stabilized with Na-caseinate and lysozyme (Na-caseinate: $F(2.9, 176)=412$, $p<0.001$; lysozyme: $F(3.1, 184)=207$, $p<0.001$).

Figure 3.3 shows the clearance of oil deposited on the front (figure **3.3A**) and back part of the anterior tongue (figure **3.3B**) for 10% (w/w) o/w emulsions stabilized with different concentrations of Na-caseinate and lysozyme at different times after expectoration. **Figure 3.3A** and **3.3B** depict the relative oil content remaining on the tongue after expectoration, *i.e.* at time 0 s the amount of oil on the tongue is 100% for all o/w emulsions. With increasing time the relative oil content ($Oil\ content\ (\%) = \frac{oil\ fraction_{t=x}}{oil\ fraction_{t=0s}} \times 100$ averaged over n=20 subjects and 3 replicate measurements) decreases to lower percentages.

Probe position had no influence on the oil clearance from o/w emulsions stabilized by Na-caseinate, showing no differences between the clearance rates of the oil deposited on the front *vs.* back of the anterior part of tongue. In contrast, the probe position significantly affected the clearance rate of the o/w emulsion stabilized with 0.2% lysozyme from 30s onwards, and of the emulsion stabilized with 5.8% lysozyme from 60s onwards. For both cases, the oil deposited on the front part of the tongue was cleared faster, *i.e.* reached lower percentages of relative oil content after shorter time periods compared to the back part of the anterior tongue. The oil clearance from the o/w emulsion stabilized by 3% lysozyme was significantly different (front *vs.* back) at time 90s ($p=0.06$) and 120s ($p=0.03$).

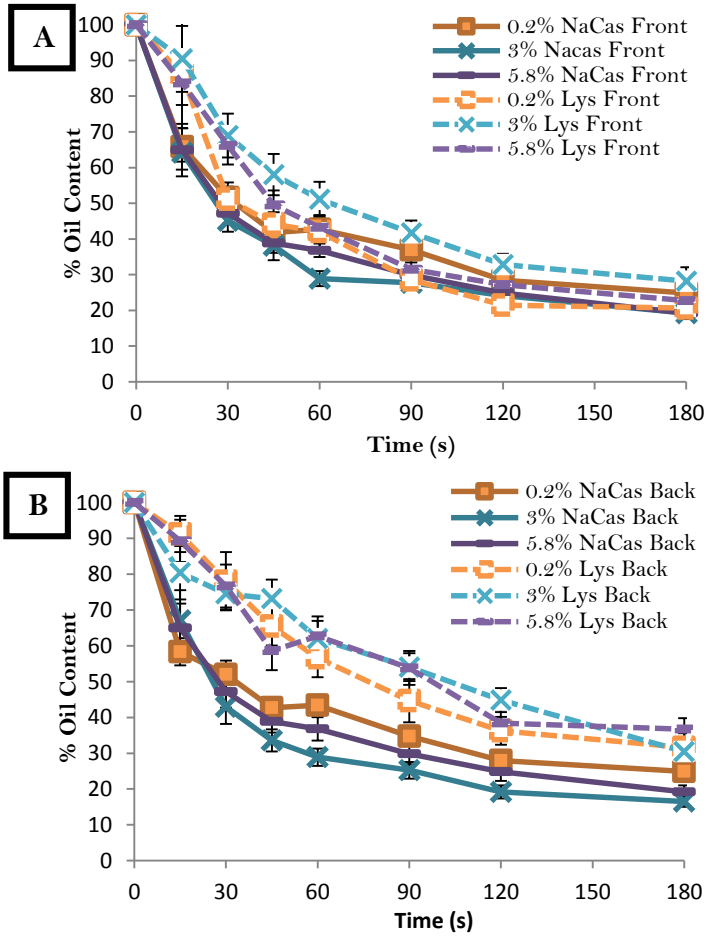

Figure 3.3. Relative oil content deposited on the front (A) and back (B) part of the anterior tongue for 10% (w/w) o/w emulsions stabilized with different concentrations of Na-caseinate (NaCas) and lysozyme (Lys) as a function of time . Each point represents the average of n= 20 subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent the standard error.

Figures 3.3A and **3.3B** show that the oil deposited on the tongue of o/w emulsions stabilized with Na-caseinate had a faster clearance compared to o/w emulsions stabilized with lysozyme, for both front and back part of the tongue. These results were significant for the back part of the tongue from 30s onwards ($p<0.05$).

The o/w emulsions stabilized with lower concentrations of Na-caseinate (0.2% w/w) had a slower clearance of oil compared with the other two o/w emulsions stabilized with Na-caseinate (3.0 and 5.8% w/w). The concentration of lysozyme in the o/w emulsion did not affect the oil clearance from the tongue.

3.2 Influence of Protein Type and Content on After-feel Perception of Oral Coatings

Figure 3.4 depicts the after-feel perception of 10% (w/w) o/w emulsions stabilized with different concentrations of Na-caseinate and lysozyme at different time points after expectorating the emulsions. O/w emulsions stabilized with Na-caseinate were perceived as significantly more intense for the after-feel attributes "fatty film", "sweetness" and "creaminess" ($p<0.001$) than emulsions stabilized with lysozyme.

The intensity of "fatty film" was significantly influenced by sample (*i.e.*, 0.2, 3.0, 5.8% lysozyme and 0.2, 3.0, 5.8% Na-caseinate) [$F(2.6, 152)=9.4$, $p<0.001$], time [$F(2.6, 154)=346$, $p<0.001$] and sample x time [$F(8.2, 484)=7.8$, $p<0.001$]. Lysozyme concentration did not affect the perception of "fatty film" after-feel attributes of o/w emulsions stabilized with lysozyme. A trend was observed with higher concentration of Na-caseinate concentration leading to higher "fatty film" after-feel perception. For the attribute "sweetness" the effects of sample (*i.e.*, 0.2, 3.0, 5.8% lysozyme and 0.2, 3.0, 5.8% Na-caseinate) [$F(2.6, 157)=21.5$], time [$F(2.1, 121)=446$] and sample x time [$F(11.9, 702)=7.8$] were significant at $p<0.001$. The same was observed for the attribute "creaminess" (sample (*i.e.*, 0.2, 3.0, 5.8% lysozyme and 0.2, 3.0, 5.8% Na-caseinate) [$F(3.6, 211)=65.9$], time [$F(2.2, 127)=464$, sample x time [$F(9.8, 577)=13.5$]). The higher the concentration of Na-caseinate in the emulsion, the higher the perceived creaminess. No differences were found between the o/w emulsions stabilized with varying amounts of lysozyme.

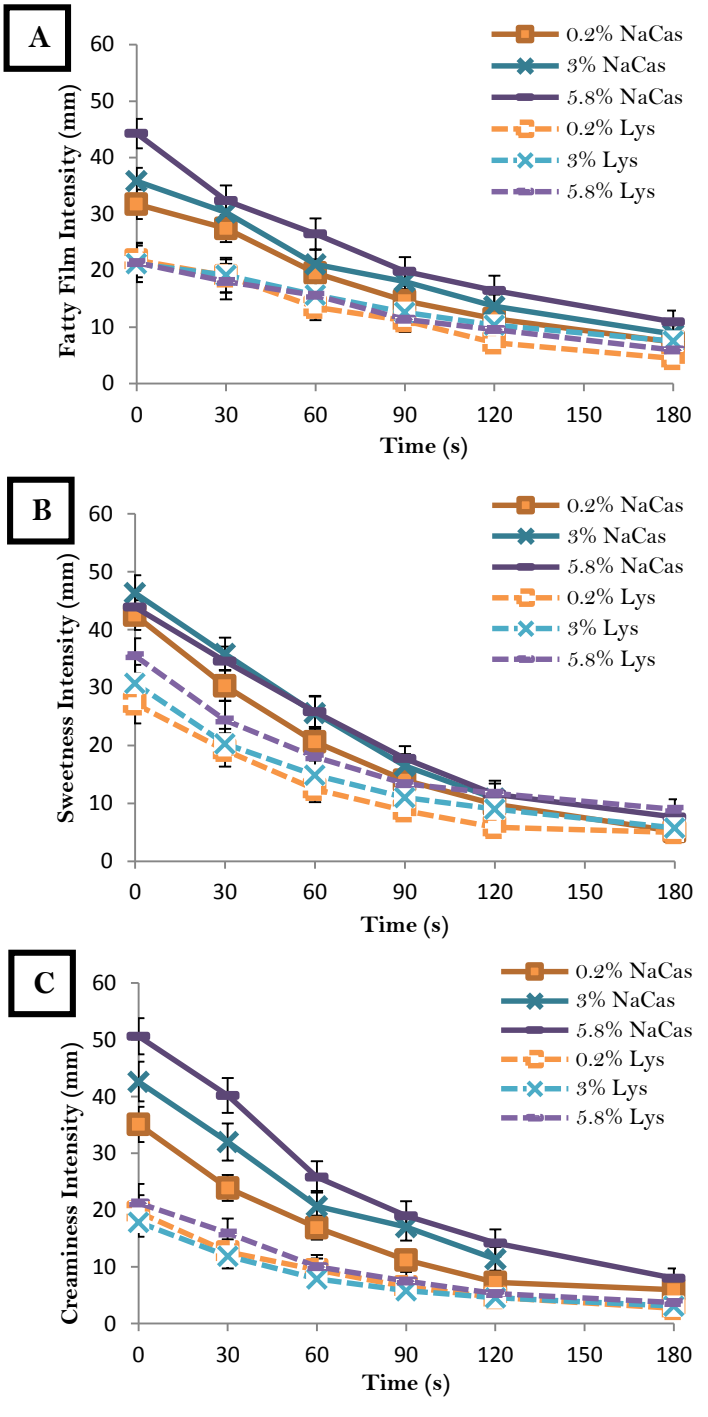

Figure 3.4. Intensity of after-feel attributes Fatty film (A), Sweetness (B) and Creaminess (C) for 10% (w/w) emulsions differing in emulsifier type (Na-caseinate (NaCas) and lysozyme (Lys))

and content as a function of time after expectoration. Each point represents the average of n= 20 subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent the standard error.

3.3 Influence of Viscosity on Clearance of Oral Coatings

Oil Deposition on Tongue Immediately after Expectoration (t=0s)

Figure 3.5 shows the oil fraction deposited on the front and back part of the anterior tongue immediately after expectoration (t=0s) of 10% (w/w) o/w emulsions stabilized with 3% (w/w) Na-caseinate differing in xanthan gum concentration. Sample (*i.e.*, 0, 0.05, 0.2 and 0.5% xanthan gum) [$F(2.3, 133)=137$, $p<0.001$] and probe position [$F(1,59)=193$, $p<0.001$] had a significant main effect on oil fraction deposited on the tongue for emulsions thickened with xanthan gum. Pairwise comparisons revealed no significant differences between o/w emulsions containing different concentrations of xanthan, but only a significant difference between o/w emulsion without xanthan gum *vs.* o/w emulsions with xanthan gum ($p<0.001$).

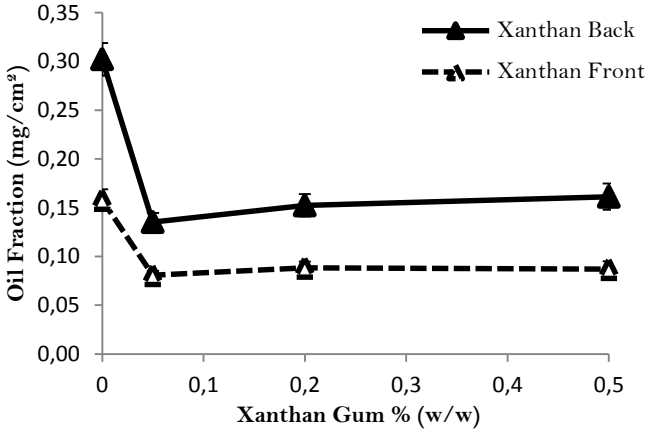

Figure 3.5. Oil fraction deposited on front and back of anterior tongue immediately after expectoration (t=0s) as a function of concentration (% w/w) of xanthan gum in the 10% (w/w) o/w emulsions stabilized with 3% (w/w) Na-caseinate. Each point represents the average of n= 20 subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent the standard error.

Immediately after expectoration of the o/w emulsions, the oil fraction deposited on the back part of the anterior tongue was significantly higher than on the front part of the tongue ($p<0.001$). For both front and back part of the anterior tongue, the o/w emulsion with no xanthan gum had a significantly higher oil fraction deposited on the tongue than o/w emulsions containing xanthan ($p<0.001$).

Clearance of Oil Deposited on the Tongue of o/w emulsions Varying in Viscosity

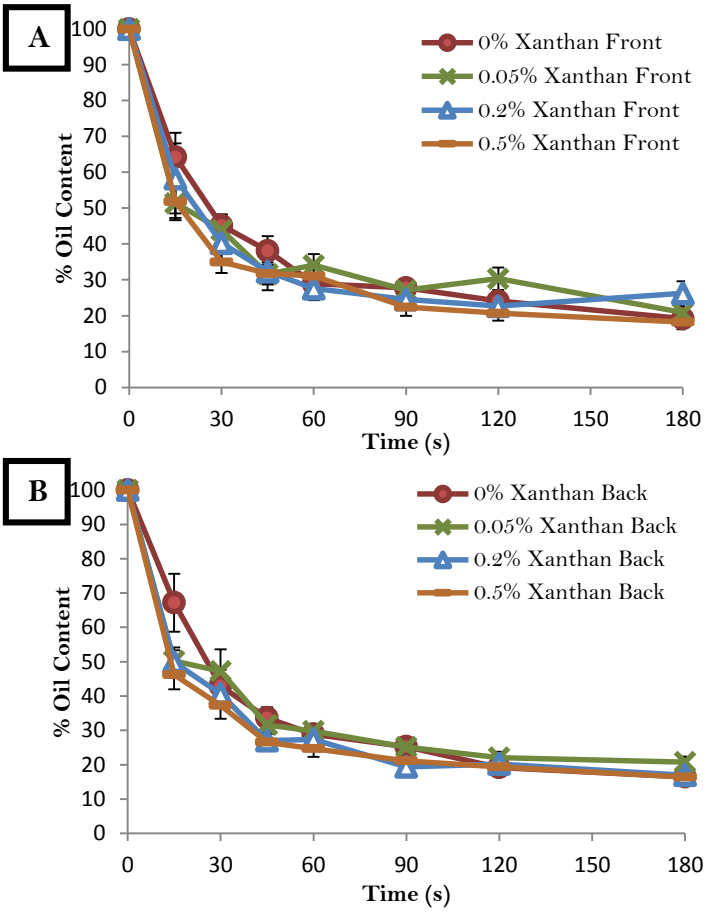

Figure 3.6. Relative oil content (%) deposited on the front (A) and back (B) part of the anterior tongue for the 10% (w/w) o/w emulsions stabilized with 3% w/w Na-caseinate differing in xanthan gum concentration as a function of time. Each point represents the average of n= 20 subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent the standard error.

Figure 3.6 depicts the relative oil content deposited on the front and back of the anterior tongue for the 10% (w/w) o/w emulsions stabilized with 3% (w/w) Na-caseinate thickened with different xanthan gum concentrations as a function of time. Time had a significant effect on oil fraction deposited on the tongue surface [$F(2.3, 136)=525$, $p<0.001$]. The oil clearance was not significantly different for o/w emulsions differing in concentration of xanthan gum ($p=0.082$). Probe position had no significant effect on the values of relative oil content ($p=0.155$).

3.4 Influence of Viscosity on After-feel Perception of Oral Coatings

Figure 3.7 depicts the intensity of the after-feel attributes of the 10% (w/w) o/w emulsions stabilized with 3% (w/w) Na-caseinate thickened with different xanthan gum concentrations.

For the attribute "fatty film" the effects of sample (*i.e.*, 0, 0.05, 0.2 and 0.5% xanthan gum) [$F(2.1, 124)=13.45$, $p<0.001$], time [$F(2.4, 140)=387$, $p<0.001$] and sample x time [$F(6.8, 398)=3.3$, $p<0.05$] were significant. The emulsions with 0.5% xanthan and no xanthan were perceived significantly higher ($p<0.05$) in "fatty film" intensity than the o/w emulsions thickened with 0.05 and 0.2% xanthan gum. The attribute "sweetness" was influenced by sample (*i.e.*, 0, 0.05, 0.2 and 0.5% xanthan gum) [$F(2.6, 153)=38.1$, $p<0.001$], time [$F(2.7, 161)=382$, $p<0.001$] and sample x time [$F(9.2, 543)=7.0$, $p<0.001$]. The o/w emulsion with no xanthan was perceived as significantly sweeter ($p<0.001$) than all other o/w emulsions. With increasing xanthan concentration after-feel sweetness tended to decrease.

The attribute "creaminess" was significantly influenced by sample (*i.e.*, 0, 0.05, 0.2 and 0.5% xanthan gum) [$F(2.6, 154)=19.3$ $p<0.001$], time [$F(2.5, 145)=471$, $p<0.001$] and sample x time [$F(8.2, 481)=4.3$, $p<0.001$]. The o/w emulsion with 0.5% xanthan was perceived significantly creamier ($p<0.05$) than all other o/w emulsions.

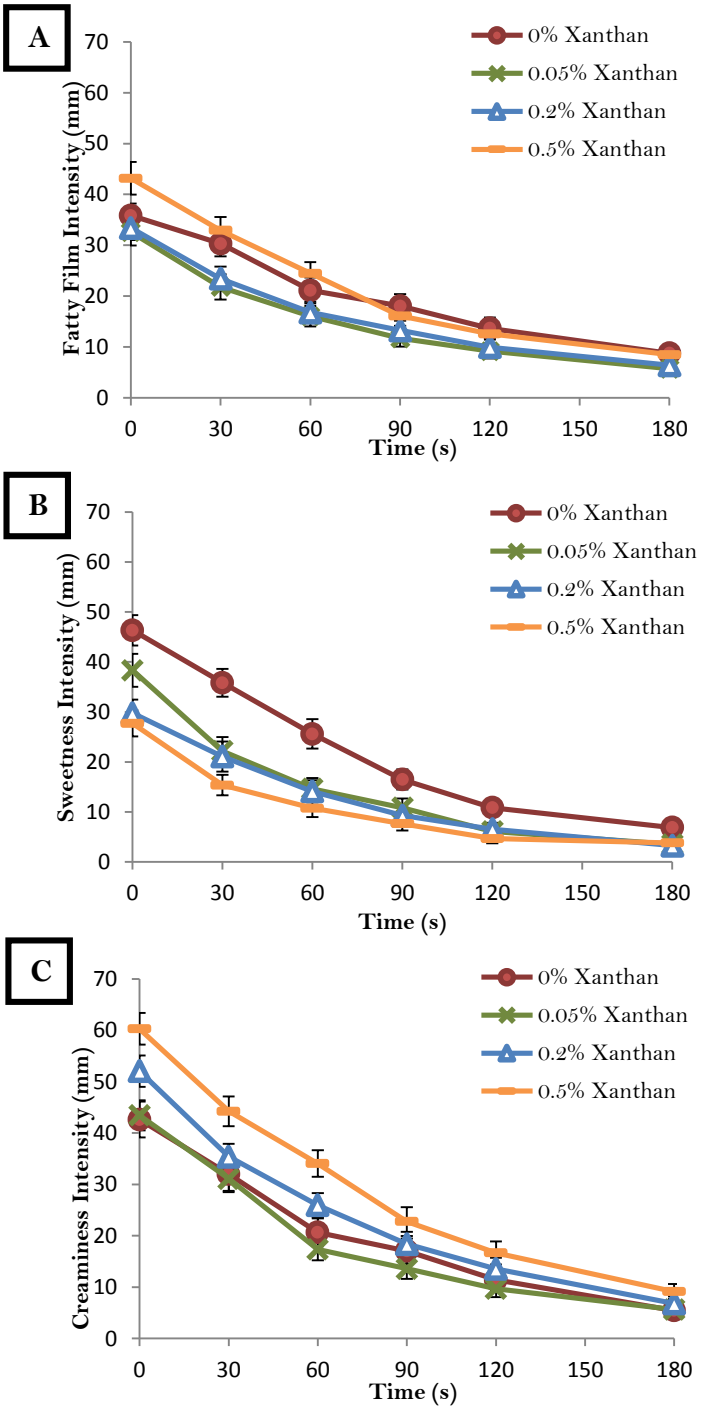

Figure 3.7. Intensity of the after-feel attributes Fatty film (A), Sweetness (B) and Creaminess (C) for o/w emulsions containing 10% (w/w) oil stabilized with 3% Na-caseinate differing in xanthan gum concentration as a function of time .

Each point represents the average of n= 20 subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent the standard error.

4. Discussion

The aims of this study were to investigate the influence of (i) protein type, (ii) protein content and (iii) viscosity of o/w emulsions on the oil fraction deposited on the tongue after expectoration of the samples, and after-feel sensory perception.

The oil fraction deposited on the back part of the anterior tongue was by a factor of around 1.3 to 1.9 times higher compared with the oil fraction deposited on the front part of the anterior tongue for all o/w emulsion tested in this study. These results are in line with previous studies that showed that the coating deposition followed a similar spatial variation (Camacho *et al.*, 2014, Pivk *et al* 2008b). It has been suggested that the spatial variation in oil deposition on the tongue is due to differences in the morphology of the tongue and/or movement of the tongue while and after orally processing the samples.

4.1 Influence of Protein Type and Content on Clearance of Oral Oil Coatings

Light microscopy revealed the microstructure of the 10% (w/w) o/w emulsions stabilized with different concentrations of Na-caseinate and lysozyme before and after mixing with human saliva. All images were taken with o/w emulsions at neutral pH, at room temperature and were mixed with the same batch of human saliva. The o/w emulsions stabilized with higher concentrations of Na-caseinate (3.0 and 5.8% w/w) showed aggregation likely due to depletion flocculation due to unadsorbed protein at high concentrations (Dickinson *et al.*, 1997a). The flocculation properties of o/w emulsions stabilized with Na-caseinate were previously shown to be comparable to micelle induced depletion in surfactant-based systems (Dickinson *et al.*, 1997a,b). As the unadsorbed protein concentration is increased the flocculation is also expected to increase. This

flocculation is weak and reversible (Dickinson *et al.*, 1997a,b). The o/w emulsions stabilized with Na-caseinate showed very limited changes in the emulsion microstructure upon mixing with human saliva. No aggregation was observed upon mixing with human saliva for o/w emulsions stabilized by 0.2% Na-caseinate. This is in agreement with the hypothesis that negatively charged proteins result in negatively charged emulsion droplets, which do not interact with salivary biopolymers (Silletti *et al.*, 2007).

The o/w emulsions stabilized with lysozyme showed no aggregation, even at higher concentrations (3.0 and 5.8% w/w). This is possibly due to the fact that lysozyme is a globular protein, and when in excess concentration to cover the oil droplets it can create multi-layers of adsorbed protein around the emulsion droplet (Hunt *et al.*, 1994). Lysozyme is known to form complexes with salivary proteins (Silletti *et al.*, 2007). The complexes are formed by electrostatic interactions between the positively charged lysozyme and the negatively charged salivary biopolymers. The complex formation is responsible for the irreversible flocculation of the lysozyme emulsions (Silletti *et al.*, 2007). Figure 3.1 shows that the lysozyme stabilized o/w emulsions (0.2, 3.0 and 5.8% lysozyme) used in this study formed large agglomerates upon mixing with saliva. *In vivo* fluorescence measurements showed that increasing the concentration of lysozyme lead to smaller oil fractions deposited on the tongue immediately after expectoration of the o/w emulsion (t=0s, see figure 3.2). It is possible that with higher concentrations of unadsorbed lysozyme more and larger agglomerates are formed upon mixing with saliva in the oral cavity. Previous studies found that starch granules were cleared faster from the tongue than xanthan gum (de Jongh *et al.*, 2007). They hypothesized that the larger starch granules might be too large to enter the voids between the papillae on the tongue and therefore were washed away easier(de Jongh *et al.*, 2007). As lysozyme forms aggregates, it is plausible that the aggregates were too large to enter the voids between the papillae during oral processing. When spitting out the o/w emulsions, it is likely that the formed

complexes were spat out as well. On the other hand, there were no differences on oil fraction between the o/w emulsions stabilized with Na-caseinate. The depletion agglomerates of caseinate micelles are weak and reversible. It is likely that due to the constant stirring of the o/w emulsions prior to in mouth processing and to in mouth shear, these agglomerates were destroyed and thus did not have an effect on the oil deposition in mouth.

The oil coatings formed by lysozyme stabilized o/w emulsions had a slower oil clearance rate from the tongue than oil coatings formed by Na-caseinate stabilized o/w emulsions. Complex formation between salivary proteins and lysozyme can take place in the saliva fluid as well as with the mucous layer (van Aken *et al.*, 2007). Complex formation with the mucous layer might slow down clearance. On the other hand, the coating formed by the Na-caseinate emulsions is likely to have no or very little binding with the mucous layer and as such, easier to be washed away by saliva and rubbing against palate.

No significant differences were found between the o/w emulsions stabilized with different concentrations of Na-caseinate. However, oil clearance tended to be slower at lower Na-caseinate concentrations. We hypothesized that excess protein has a blending effect with saliva resulting in faster clearance of oil deposits from the tongue surface. The blending effect could be the underlying mechanism responsible for the observed trend. However, this effect depends on the behavior of the protein in mouth. In the case of the o/w emulsions stabilized with lysozyme no trend was observed indicating as dominant effect the behavior of the protein with saliva and to a lesser extent the concentration of protein in the emulsion.

The after-feel perception of lysozyme stabilized o/w emulsions was perceived as less intense compared to Na-caseinate stabilized o/w emulsions. As the clearance of the oil fraction deposited on the tongue for the o/w emulsions stabilized with lysozyme was slower than for o/w emulsions stabilized with Na-caseinate, the oil fraction deposited on tongue was larger at almost every measured time point (data not shown). It would be expected that the oil coatings left by the lysozyme stabilized o/w emulsions would be perceived as fattier and creamier since they

effectively have more oil deposited on the tongue than Na-caseinate stabilized o/w emulsions. It is known though that lysozyme provokes an astringent and dry sensation in the mouth, which are sensations that are likely to reduce the intensity of the attributes "fatty film" and "creaminess". The flocculation of lysozyme stabilized o/w emulsions was found to have similarities to saliva flocculation observed by addition of tannins. The flocculation with proteins in saliva and mucous layer reduces the lubrication properties of saliva and increases friction in mouth which leads to astringent and rough after-feel (Vingerhoeds *et al.*, 2009). The after-feel sensations seem to be complex and not a sole function of the actual amount of oil deposited on the tongue.

4.2 Influence of Viscosity on Clearance of Oral Oil Coatings

A decrease in oil fraction deposited on the tongue immediately after expectoration of o/w emulsions when xanthan gum was present compared to o/w emulsions without xanthan was observed. This decrease was independent of xanthan concentration added to thicken the o/w emulsion. This suggests that the matrix created by xanthan is likely to capture the oil droplets and create a film which does not allow the oil droplets to enter the voids between the papillae, and thus creating less oil deposition compared to emulsions without xanthan gum. Other studies have also shown that the addition of polysaccharides, such as guar gum, to emulsions reduces oil retention on the tongue surface (Vingerhoeds *et al.*, 2009). Our results show that there is no significant difference in oil clearance dynamics depending on xanthan concentration. Furthermore, no influence on the fat clearance kinetics of different types of thickeners was found (de Jongh *et al.*, 2007). It is interesting to note that "fatty film" perception was higher for o/w emulsions with the highest concentration of xanthan (0.5%). Physically, the oil deposition for both emulsions is very different with the emulsion with no xanthan having the highest oil fraction deposited on tongue. This suggests that xanthan gum might create a lubricating layer on the tongue, thereby decreasing the friction and increasing fatty after-feel. This lubrication effect is likely to dependent on the

concentration of thickener. In accordance, "creaminess" was perceived as significantly higher for the 0.5% xanthan o/w emulsions compared to the o/w emulsions with lower concentrations of xanthan. All o/w emulsions with added xanthan were perceived as significantly less sweet compared with the 3% Na-caseinate stabilized o/w emulsion without xanthan. This was expected as it is known that increasing viscosities in liquid foods can decrease taste perception (Walker *et al.*, 2000, Hollowood *et al.*, 2002, Cook *et al.*, 2003). Different explanations for taste suppression caused by thickeners were reported: kinetic and perceptual. Kinetic explanations suggest a reduction of the tastant release and diffusion rates in-mouth due to the binding of tastants to the thickener, or due to inhibition of the tastants´ transport due to the hydrocolloid chains (Ferry *et al.*, 2006, Baines *et al.*, 1987, Cook *et al.*, 2005). Perceptual interactions were also demonstrated to contribute to the effect of taste perception due to texture differences, caused by cross-modal interaction between tactile and taste signals (Burseg *et al.*, 2011).

In conclusion, considerable differences were found in oil fraction deposited on the tongue and after-feel perception between o/w emulsions stabilized by Na-caseinate which do not aggregate under in mouth conditions and o/w emulsions stabilized by lysozyme which aggregate under in mouth conditions. The irreversible agglomeration of the o/w emulsions stabilized with lysozyme caused a slower oil clearance of the oral oil coating from the tongue compared to o/w emulsions stabilized with Na-caseinate. The increase of unadsorbed lysozyme in the o/w emulsion might have created larger agglomerates that were easier expectorated creating smaller depositions of oil immediately after expectoration than the Na-caseinate stabilized emulsions. The after-feel of o/w emulsions stabilized with Na-caseinate was perceived as creamier, fattier and sweeter than the o/w emulsions stabilized with lysozyme. The addition of xanthan gum to Na-caseinate stabilized o/w emulsions decreased the deposition of oil on tongue although o/w emulsions with higher concentrations of xanthan had a similar or higher fatty and creamy after-feel perception than o/w emulsions without

xanthan. We conclude that the after-feel perception of o/w emulsions is complex and that the physical deposited oil on the tongue contributes to it as well as factors such as the behavior of proteins in mouth and the presence of thickeners.

Abbreviations Used:

NaCas: Na-caseinate (Sodium caseinate), Lys: Lysozyme, xanthan: xanthan gum, o/w: oil in water.

Formation Dynamics of Oral Oil Coatings and its Effect on Subsequent Sweetness Perception of liquid Stimuli

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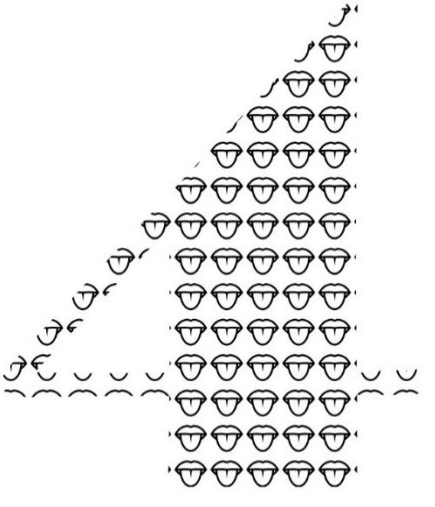

Abstract

Knowledge on formation of oral coatings and their influence on subsequent taste perception is necessary to understand possible taste masking effects by oil coatings. This study investigated (a) the dynamics of the formation of oral oil coatings formed by o/w emulsions and (b) the effect of oral oil coatings on subsequent sweetness perception of sucrose solutions. *In vivo* fluorescence was used to quantitate the oil fraction deposited on the tongue after oral processing o/w emulsions for different times. A trained panel evaluated sweetness perception of sucrose solutions after orally processing the emulsions. Oil fraction reached its maximum value within the first 3 s of oral processing. Oil fraction did not significantly affect subsequent sweetness perception of sucrose solutions. We suggest that the oil droplets deposited on the tongue did not form a hydrophobic barrier that is sufficient to reduce the accessibility of sucrose to taste buds.

Keywords: oral coatings, formation, sweetness, emulsion, perceptual, *in vivo* fluorescence

1. Introduction

Oral coatings are residues of foods that remain on the oral mucosa after consumption. Oral oil coatings have been suggested to influence subsequent taste perception (Lynch *et al.*, 1993, Valentová and Pokorný,1998, Ahn *et al.*, 2002). To better understand the mechanisms underlying the effect of oral oil coatings on taste perception, the effect of oil and tastant stimulus can be dissociated e.g. by analyzing the effects of oil containing products on subsequent taste perception. Previous studies used time-intensity profiling to assess the effect of an oral oil coating formed by either sunflower or coconut oil on subsequent taste intensity of sweet, salt, sour or bitter tasting semi-solid gelatin gels (Lynch *et al.*, 1993). The presence of an oil coating lead to a decrease of the maximum taste intensity. It was suggested that the physical interference of the hydrophobic oil coating with the hydrophilic tastants reduces the accessibility of the tastants to the taste buds leading to a decrease in taste intensity (Lynch *et al.*, 1993). Valentová and Pokorný (1998) studied the effect of oral oil coatings formed by sunflower oil on subsequent taste perception of liquid tastant stimuli (Valentová and Pokorný, 1998). It was found that the oil coating reduced the subsequent intensity of sweetness and bitterness demonstrating that the taste suppression observed by previous studies in semi-solid gels (Lynch *et al.*, 1993) is also observed in liquid stimuli for sweetness and bitterness. Contrariwise, the intensity of sourness and saltiness were not affected by the oral oil coating. It was hypothesized that the different effects of oral oil coatings on subsequent taste perception were caused by differences in the chemical structure between the tastants. It was suggested that sucrose and quinine are relatively large molecules and their diffusion through the lipid layer might have been suppressed. Conversely, the ions of Na^+ and H^+ responsible for salty and acidic taste are relatively small and could pass through the lipid layer to the taste receptors more easily (Valentová and Pokorný, 1998). These two studies (Lynch *et al.*, 1993, Valentová and Pokorný, 1998) have in common that the tongue surface was first coated by bulk oils and then

subsequently taste perception was determined. Consumers usually do not drink bulk oils but use them for food preparations. Therefore, it is not known whether the reported effects of oil coatings originating from oral processing of bulk oils on taste can be generalized to oil containing beverages and foods consumed in day-to-day life.

Valentová and Pokorný (1998) investigated the effect of oral coatings formed by commercial oil/water emulsions (yogurt dressing, soup and mayonnaise) on subsequent sweetness and bitterness perception. A decrease in intensity of both taste modalities was found for all tested commercial o/w emulsions (Valentová and Pokorný, 1998). Ahn *et al.*, (2002) studied the effect of different beverages on subsequent taste perception of tastant solutions using time-intensity profiling. Consumption of milk lowered subsequent perception of sweetness and saltiness of tastant solutions. This effect was found to be higher in full fat compared to skimmed milk. It was suggested that the lipids present in the milk resulted in an oil coating on the tongue which was greater for full fat than for skimmed milk, thus creating a thicker hydrophobic barrier leading to a larger taste intensity suppression (Ahn *et al.*, 2002). However, the amount of oil or fat present on the tongue was not quantified. Further, the beverages used to create the oral coatings have an inherent taste which complicates the dissociation between the taste intensity suppression possibly being caused by the physical oil barrier of the coating or by a perceptual taste-taste interaction between the beverage and the tastant stimulus.

These studies suggested that a physical hydrophobic barrier formed by the fat coating on the tongue surface hinders the migration of hydrophilic tastants through the hydrophobic barrier leading to less tastants molecules reaching the taste buds to trigger a taste response consequently leading to a decline in taste intensity. The hypothesis might hold true when oil coatings form a sufficiently thick film covering the tongue surface which might be the case after orally processing bulk fats and oils. Perceptual effects such as taste-taste, flavor-taste or texture-taste interactions between the food used to coat the tongue and the

subsequently provided tastant stimulus might have an impact when complex, multi-component commercial products are consumed. To better understand the mechanisms underlying taste suppression due to oil deposition on the tongue surface, it is important to study oral coatings formed by foods with low taste and flavor intensity at realistic oil contents as they occur in foods. Besides the effect of oral coatings on subsequent taste perception, several studies focused on the factors affecting the dynamics of physical clearance of oral coatings after stimulus expectoration (Pivk *et al.*, 2008b, Camacho *et al.*, 2014 and 2015, Prinz *et al.*, 2006). Little is known about the dynamics of the formation of oil deposits on the tongue while drinking.

The first aim of this study was to quantitate the dynamic formation of oil deposits on the tongue surface formed by o/w emulsion differing in oil content from 1 to 20% (w/w). For this purpose one sip (20 mL) of o/w emulsion containing 1, 10 and 20% (w/w) oil and 3.2% (w/w) Na-caseinate was processed in mouth for different times ranging from natural consumption time (3 s) to times which were hypothesized to create maximum oil deposition on the tongue (15 s). *In vivo* fluorescence measurements were used to quantitate the oil fraction deposited on the tongue at each time point. The second aim of this study was to investigate the effect of oil deposits on the tongue surface formed by o/w emulsions on subsequent sweetness perception of sucrose solutions. A trained panel was used to evaluate sweetness intensity of 4% (w/w) sucrose solutions after oral processing of the o/w emulsions for different times.

We hypothesize that oil fraction deposited on the tongue increases with increasing oral processing time until, at longer oral processing times, the amount of oil deposited on the tongue reaches a plateau. We hypothesize that oral processing of o/w emulsions with relatively low oil content does not lead to the formation of a hydrophobic barrier that is sufficient to reduce the accessibility of hydrophilic tastants to the taste buds, and consequently does not influence subsequent taste perception.

2. Materials and Methods

2.1 Materials

Sunflower oil (Albert Heijn, purchased from local retailer), Na-caseinate (Excellion sodium caseinate S, DMV International, The Netherlands, protein content: 91%) and tap water were used for the preparation of the emulsions. Curcumin (7% w/w curcumin dissolved in propylene glycol and polysorbate; L-WS; Sensient, Elburg, The Netherlands) was added to the o/w emulsions as a hydrophobic fluorescent dye. Sucrose (Van Gilse Kristalsuiker, purchased from local retailer) and bottled mineral water (C1000, purchased from local retailer) were used for the preparation of the aqueous sucrose solutions. All ingredients were food grade.

2.2 Preparation and Characterization of o/w Emulsions and Sucrose Solutions

All o/w emulsions were prepared in a food-grade environment. The aqueous phase of the o/w emulsions consisted of 3.2% (w/w) Na-caseinate solution. The aqueous phase was prepared by dissolving Na-caseinate in tap water at room temperature. The aqueous phase was then pre-homogenized with the sunflower oil (1, 10 and 20% w/w) using an Ultra Turrax (IKA® RW 20 Digital) at 4000 min-1 for 2 minutes and homogenized with a two-stage homogenizer (Niro-Soavi S.p.A. NS1001L2K) at pressures between 200 and 350 bar. All o/w emulsions had a neutral pH. The emulsion droplet size distribution was measured by light scattering (Mastersizer 2000, Malvern Instruments) in duplicate using the refractive index of sunflower oil (1.469). The average Sauter diameter of the emulsion droplets $d_{3,2}$ was determined for each o/w emulsion (average $d_{3,2}$ = 0.70 μm) accounting for differences in batches (one batch of each o/w emulsion was made per week). Emulsions were stored after preparation in a refrigerator at 4°C and used for further studies no later than three days after preparation. Curcumin

solutions were added to the o/w emulsions under magnetic stirring before consumption (Camacho *et al.*, 2014).

Sucrose solutions varying in concentration were prepared by dissolving sucrose in water under stirring at room temperature. For the screening sessions (to find suitable subjects for the experiments), a range of 2 to 8% (w/w) sucrose solutions was prepared. For the training sessions of selected subjects, 2.5, 3.0, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 5.0 and 5.5% (w/w) sucrose solutions were prepared. For the experiment addressing the second objective of this study, a 4% (w/w) sucrose solution was prepared.

2.3 Panel Selection

Twenty-eight subjects (21 female, 7 male, age 25.5 ± 5.2 years) were invited for a screening session of one hour. The screening session started with a ranking test in which subjects were asked to taste four sucrose solutions (2, 4, 6 and 8% (w/w) sucrose) and rank them in order of increasing sweetness. This was followed by a second ranking test in which subjects were asked to taste five sucrose solutions (2, 3, 4, 5 and 6% (w/w) sucrose) and rank them in order of increasing sweetness. The presentation order of stimuli in the screening session was randomized in both ranking tests. All subjects performed both ranking tests in duplicate. The third test of the screening session was a Duo-Trio test in which the subjects were asked to first taste the reference stimulus (4% (w/w) sucrose solution) and then choose out of two stimuli the one that was most similar to the reference stimulus. The sucrose concentrations of the two stimuli were either 3 and 4% (w/w) or 4 and 5% (w/w). The test was performed in duplicate by all subjects. Presentation order and position were randomized over subjects in the Duo-Trio test. The performance of each subject on the screening session was determined. The correct answers given were weighted for the difficulty of the task, *i.e.* 1 fold for the first ranking test, 2 folds for the second ranking test and the Duo-Trio test. This resulted in an individual performance score for each subject. Based on the

performance score 15 subjects (12 female and 3 male, age 23.6 ± 3.5 years) with the highest performance score were selected to participate in the study from the 28 subjects participating in the screening session.

The screening session ended with a consumption time measurement. All 28 subjects were asked to measure the drinking time between ingesting and swallowing a sip (20 mL) of full fat milk (FrieslandCampina, The Netherlands). A stopwatch was used by each subject. This test was performed in triplicate. The average oral processing time of 20 mL of full fat milk was found to be 3.3 ± 0.2 s.

All subjects signed a consent form and received financial compensation for their efforts.

2.4 Panel Training

The panel (n=15) was trained on sweetness intensity evaluation of sucrose solutions to ensure that the panel is able to discriminate sweetness intensity of stimuli varying in sucrose concentration by less than 0.4% (w/w) sucrose relative to a reference stimulus (4.0% (w/w) sucrose). The panel was trained in six sessions of one hour twice a week for three weeks. Each training session started with individual feedback on the performance during the previous training session to improve panel performance. The panel used a 150 mm visual analogue scale (VAS) scale during all training sessions. The training sessions involved group discussions during which the panel was first introduced to the 150 mm VAS, tasting instructions, reference stimuli and stimuli with different sweetness intensities. The use of the scale and its suitability were discussed for different sucrose solutions. These group discussions were followed by individual tasting tests.

An overview of the training sessions is given in **table 4.1**. During the first training session, samples representing concentration differences of 1% (w/w) sucrose were presented to the subjects. Since the effects of a coating on sweetness

intensity were expected to be small, the sucrose concentration differences between stimuli were lowered during the following training sessions ending with a concentration difference of 0.1% (w/w) (**Table 4.1**). During the second training session, samples were assessed in comparison to 3, 4 and 5% (w/w) sucrose. In the first two sessions subjects were allowed to take as many sips as they wanted of the reference and the test stimulus in order to score the sweetness. From the third training session onwards, the reference stimuli were presented to the panel. The end points of the 150 mm VAS scale were defined as the sweetness of 2.5% and 5.5% (w/w) sucrose solutions in order to demonstrate the limits of the scale and to prevent end-use avoidance. Furthermore, the 4% (w/w) sucrose solution was termed as "reference". The panel was instructed to score this reference in the middle of the scale. The panel was trained to evaluate the samples with respect to how different the sweetness intensity of each sample is compared to the reference. From the third session onwards, the panel was instructed to first taste the reference, clean the palate with water, then taste the test sample only once and rate the sweetness perception on the scale. During the fifth and sixth training sessions, the subjects were introduced to and familiarized with the procedure of the final experiments (oral processing protocol, different o/w emulsions and different oral processing times). The panel was instructed to taste the reference (4% (w/w) sucrose), take a sip of water, ingest the o/w emulsion, spit it out after a specified time period, taste the sweet sample (only once) and rate the sweetness of this stimulus. The panel was trained and instructed to focus on the sweetness perception of the sample.

Table 4.1. Description of training sessions. Concentration are sucrose concentrations, unless specified.

Training session	Method	Instructions	Concentrations of stimuli (% w/w)	Replicates
Training 1	150 mm VAS scale (*not sweet at all – extremely sweet*)	Taste the sample (as many sips as needed)	3, 4, 5	Duplicate
	Duo-Trio	Choose the sample that is most similar to the reference sample	Reference: 4 Other samples: 3.5, 4.5	Duplicate
Training 2	150 mm VAS scale (*not sweet at all – extremely sweet*)	Taste the sample (as many sips as needed) in comparison to the 3, 4 and 5%	2.5, 3.5, 4, 4.5, 5.5	Duplicate
	Paired comparison	Choose the sample that is most sweet	3.6, 4 and 4, 4.4	Duplicate
Training 3	150 mm VAS scale (*little sweet – very sweet*)	Taste the sample (only once) in comparison to the reference sample (4%)	3.6, 4, 4.4	Duplicate
			3.7, 4, 4.3	Duplicate
	Paired comparison	Choose the sample that is most sweet	3.8, 4 and 4, 4.2	Duplicate
Training 4	150 mm VAS scale (*little sweet – very sweet*)	Taste the sample (only once) in comparison to the reference (4%)	3.7, 4, 4.3	Triplicate
			3.8, 4, 4.2	Duplicate

Training session	Method	Instructions	Concentrations of stimuli (% w/w)	Replicates
Training 5	150 mm VAS scale (*little sweet – very sweet*)	Taste the reference (4%), take a sip of water, taste the sample (only once)	3.7, 4, 4.3	Triplicate
		Taste the reference (4%), take a sip of water, process the o/w emulsion (t=30s), expectorate, taste blind 4% sucrose and score sweetness relative to the reference	4 o/w emulsions: 1,10 and 20	-
Training 6	150 mm VAS scale (*little sweet – very sweet*)	Taste the reference (4%), take a sip of water, process the o/w emulsion (t=3, 6, 9, 15s), expectorate, taste blind 4% sucrose and score sweetness relative to the reference	4 o/w emulsions:1,10 and 20	-
Extra session (1)	150 mm VAS scale (*little sweet – very sweet*)	Taste the reference (4%), take a sip of water, taste the sample (only once)	3.8, 4, 4.2	Triplicate
Extra session (2)	150 mm VAS scale (*little sweet – very sweet*)	Taste the reference (4%), take a sip of water, taste the sample (only once)	3.9, 4, 4.1	Triplicate

2.5 *In vivo* Fluorescence Measurements to determine Oil Fraction deposited on Tongue

The method used in the present study has been previously described in detail (Camacho *et al.*, 2014). Fluorescence measurements were made with a single point measurement (Flouorolog Instruments SA Inc, HORIBA Jobin Yvon, Longjumeau, France) at an excitation wavelength of 440 nm, an emission wavelength of 495 nm with a slit width of 0.95 mm and a measurement time of 0.1 s. A fluorescence remote read fiber optic probe was used to measure on tongue surfaces (*in vivo* for determination of oil fraction, and *ex vivo* for calibration curves). The probe was put perpendicularly to the anterior back part of the tongue surface and with slight pressure to avoid deformation of the papillae and the coating. To convert the fluorescence intensity measured on the subjects tongue surface to the oil fraction deposited on the tongue surface, *i.e.* mass of oil per area of tongue (mg/cm^2), calibration lines (data not shown) were made following the procedure described previously (Camacho *et al.*, 2014).

2.6 Oral Processing Protocol

In order to study the formation of the oil oral coating, 20 mL o/w emulsions (1, 10 and 20% (w/w) o/w emulsion) were processed in mouth for different time periods (t =3, 4.5, 6, 9 and 15 s) and then expectorated. The different oral processing time were chosen to range from the natural drinking time of one sip of 20 mL of full fat milk (3.3 ± 0.2 s) to a consumption time that hypothetically creates maximum oil deposition on the tongue. In order to verify whether the oral processing protocol would influence the perception of the 4.0% (w/w) sucrose solution (reference), i.e. to verify whether the panel is able to score the 4.0% (w/w) sucrose solution in the middle of the VAS scale as in the training sessions, a blank coating stimulus was added to the design. The blank coating stimulus consisted of water with curcumin (in order to have a similar color as the o/w emulsions) and was processed in mouth for 9 s. The oral processing time of 9 s

was chosen as it was approximately in the middle of the range of the oral processing times of the measured o/w emulsions.

In order to assess the effect of oil deposition on the tongue on subsequent sweetness perception, the trained panel processed in-mouth the three o/w emulsions for different times or the blank stimulus at room temperature. The emulsions or the blank coating were then expectorated and the *in vivo* fluorescence measurement performed. Immediately after the fluorescence measurement which typically took around 1 to 2 s, the 4.0% (w/w) sucrose solution was tasted and sweetness intensity assessed on a continuous 150 mm VAS scale (anchored with little sweet on the left end and very sweet on the right end of the scale). Only one sip of the sucrose solution was tasted with a maximum sip size of 20 mL. The middle point of the scale (75 mm) was marked with an anchor. Sensory data was collected using EyeQuestion (version 3.11.1).

16 conditions ((3 o/w emulsions x 5 oral processing times) + 1 blank) were tested by the n=15 subjects in triplicate resulting in 48 measurements per subject. Subjects participated in eight evaluation sessions of one hour. During each session 8 stimuli were assessed by each subject. A completely randomized design was used randomizing the 48 measurements over subjects and sessions.

The panel was not allowed to drink or eat one hour before the start of a session. Prior to the first measurement, the subjects cleaned their tongue with cold water. Before the consumption of each o/w emulsion or blank coating, the subjects were asked to taste the reference (4.0% w/w sucrose) in order to re-familiarize the subjects to the reference after which they cleaned the tongue with water. This was followed by a background fluorescence measurement of the tongue. Between the fluorescent measurements the tongue was cleaned extensively to remove the oil deposited at the tongue for which crackers, a tongue scraper, and both room temperature (20 °C) and warm (40 °C) water were used.

2.7 Statistical Data Analysis

SPSS® Statistics version 21 (IBM Software, Armonk, USA) was used for the statistical analysis. Descriptive statistics were used to obtain the mean and standard error (SE). Outliers (z>2) were removed from the data. The data obtained during the training sessions were used to verify panel performance. The discriminative ability of the panel and the influence of the protocol on perception were tested by an one-way analysis of variance (ANOVA). Subject and replicate effects were verified for both the fluorescence and the sensory data by a one-way ANOVA. Fluorescence intensity data was normalized with a square root transformation. The effects of oil content (within subject factor: 1, 10, 20% w/w), oral processing time (within subject factor: 3, 4.5, 6, 9, 15 s) and the interaction on the formation of the coating were tested with a repeated-measures ANOVA. The assumption of sphericity was verified by Mauchly's test, being violated for oil content and the interaction effect. Therefore, the Greenhouse-Geisser correction was applied. The effects of oil content (within subject factor: 1, 10, 20% w/w), oral processing time (within subject factor: 3, 4.5, 6, 9, 15 s) and the interaction on sweetness were tested with a repeated-measures ANOVA. The assumption of sphericity was violated and the Greenhouse-Geisser correction was applied. When significant main effects were found, Bonferroni pairwise tests were performed. A significance level of $p < 0.05$ was chosen.

3. Results and Discussion

The aims of this study were to investigate (a) the dynamic formation of oral oil coatings formed by o/w emulsions on the tongue surface and (b) the effect of oil coatings formed by o/w emulsions on subsequent sweetness perception of sucrose solutions.

3.1 Panel Training

The results from the training sessions are shown in **figure 4.1**. The panel scored the sweetness intensity of a blind 4.0% (w/w) sucrose solution close to the middle of the 150 mm line scale (75.4 ± 2.1 mm). The discriminative ability of the panel, i.e. the minimum difference of sucrose concentration relative to the reference (4% (w/w) sucrose solution) leading to a significant difference of sweetness was determined (**Figure 4.1**). The panel assessed the sweetness of the 3.7% sucrose solution as significantly less sweet ($p < 0.001$) than the 4.0% (w/w) sucrose solution. The sweetness intensity of the 4.3% (w/w) sucrose solution ($p < 0.01$) was significantly higher than the sweetness of the 4.0% (w/w) sucrose solution. The panel did not perceive the sweetness of the 3.8% ($p = 0.118$) and the 4.2% (w/w) solution ($p = 0.168$) as significantly different from the 4.0% (w/w) sucrose solution (**Figure 4.1**). The minimum difference in sucrose concentration between stimulus and 4.0% (w/w) sucrose solution that lead to a significant difference in sweetness intensity was 0.3% (w/w).

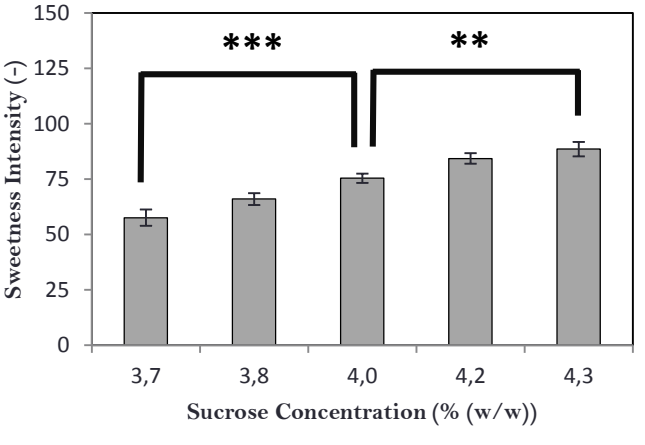

Figure 4.1. Sweetness intensity of sucrose stimuli varying in concentration rated on a 150 mm VAS scale. Each bar represents the average intensity of n=15 subjects assessing the stimuli in triplicate. Significance level is represented by ** for $p<0.01$ and *** for $p<0.001$. Error bars represent the standard error.

3.2 Dynamic Formation of Oral Oil Coatings of o/w Emulsions

Figure 4.2 depicts the oil fraction deposited on the anterior back part of the tongue for 1, 10, 20% (w/w) o/w emulsions after orally processing the emulsion for different times. A significant main effect of oil content on the oil fraction deposited on the tongue ($F[1.4, 63.4] = 323.7$ $p < 0.001$) was found with a higher oil content of the o/w emulsions resulting in a higher oil fraction deposited on the tongue surface. All the o/w emulsions differed significantly from each other at every oral processing time point ($p < 0.001$).

It was hypothesized that the oil fraction deposited on the tongue increases with increasing oral processing time until, at longer oral processing times, the amount of oil deposited on the tongue reaches a plateau. The oil fraction deposited on the tongue tended (not significant) to be influenced by oral processing time ($p = 0.057$) (Figure 2). No significant differences in oil fraction deposited on the tongue were observed between o/w emulsions orally processed for $t = 3$ s and longer oral processing times ($t = 4.5$, 6, 9 and 15 s). This demonstrates that a saturation with oil deposited on the tongue was reached already after an oral processing time of $t = 3$ s for all emulsions tested. The dynamics of the oil coating formation is similar for the three emulsions studied. This fast saturation of oil deposited on the tongue surface suggests that the o/w emulsions contain oil droplets which can easily adhere to the tongue surface. This suggests that within the natural drinking time of one sip of 20 mL of a liquid stimulus (3.3 ± 0.2 s for full fat milk), the tongue surface is already coated with the drink's oil which can adhere to the tongue. We speculate that the observations of previous studies of for instance Pivk *et al.*, (2008) (study used samples of 0.5mL-16 mL of bulk MCT oil) and Camacho *et al.*, (2014, 2015) (study used samples of 20 mL as is the case of this study) which use an oral processing time of 30 s to investigate oil deposition on the tongue, are likely to be similar to results which would have been obtained with shorter oral processing times of less than 5 s.

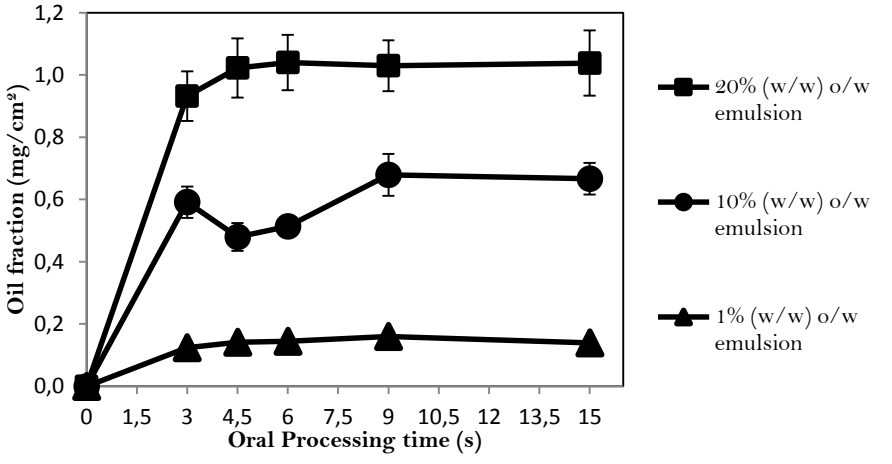

Figure 4.2. Oil fraction deposited on the back part of the anterior tongue of 1, 10 and 20% (w/w) o/w emulsions processed orally for different time periods. Each point represents the average of n=15 subjects assessing the stimuli in triplicate. The error bars represent the standard error.

3.3 Influence of Oil Coatings on subsequent Sweetness Perception

The influence of the oral processing protocol during the fluorescence measurements on the evaluation of sweetness intensity of the sucrose samples was verified by adding a blank coating sample (water with curcumin) to the experiment. The sweetness intensity of the 4.0% sucrose sample after consuming the blank (76.7 ± 2.5 mm) did not differ significantly from the sweetness intensity of the 4% sucrose solution without consuming the blank (75.4 ± 2.1 mm), ($p = 0.696$). This indicates that the oral processing protocol did not affect the panel performance and further, the addition of curcumin did not affect sweetness.

Different oil fractions deposited on the tongue (**Figure 4.2**) after different oral processing times did not significantly affect subsequent sweetness intensity ($p = 0.294$) (**Figure 4.3**). Sweetness intensities of the 4.0% (w/w) sucrose stimulus, after orally processing the different o/w emulsions for different times, ranged from 71.1 ± 2.1 to 77.4 ± 2.2 mm. Sweetness intensity was not correlated with the oil fraction deposited on the back part of the anterior tongue (**Figure 4.4**).

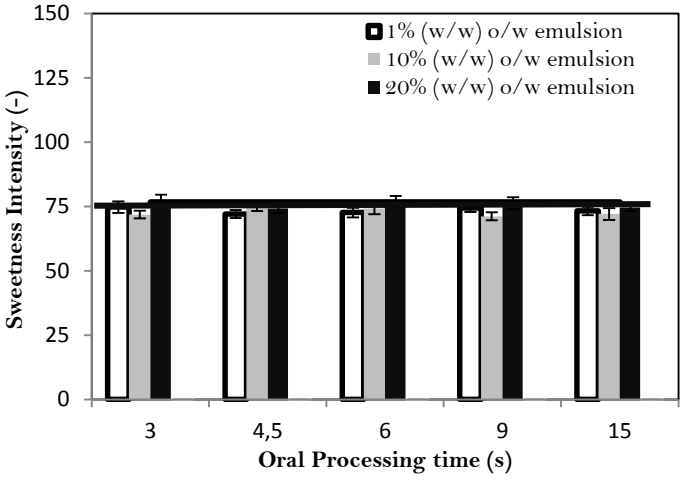

Figure 4.3. Sweetness intensity after consumption of 1, 10 and 20% (w/w) o/w emulsions for different oral processing times (t=3, 4.5, 6, 9 and 15s). Each bar represents the average value of n=15 subjects assessing the stimuli in triplicate. The error bars represent the standard error. The line represents the average sweetness intensity of the blank sample (*i.e.* water).

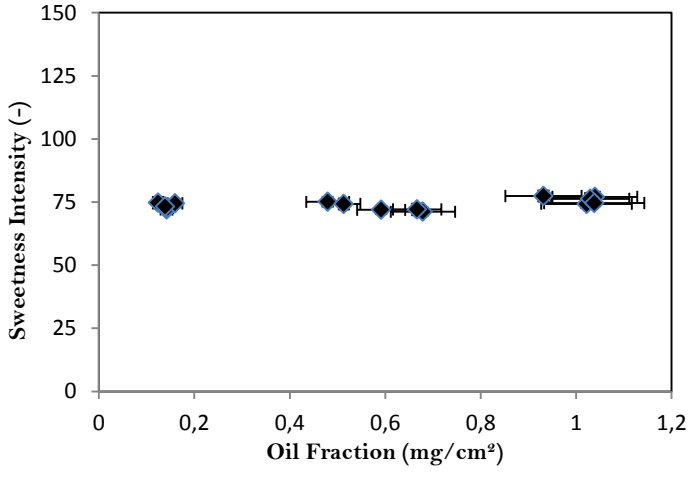

Figure 4.4. Oil fraction deposited on the back part of the anterior tongue as a function of sweetness intensity for 1, 10 and 20% (w/w) o/w emulsions orally process for different times (t=3, 4.5, 6, 9 and 15s). Each point represents the average of n=15 subjects assessing the stimuli in triplicate. The error bars represent the standard error.

We suggest that by orally processing o/w emulsions ranging in oil content from 1 to 20% (w/w), the oil fraction adhering to the tongue surface is not sufficient to form a homogeneous film of oil covering the tongue. We suggest that the oil droplets deposited on the tongue do not form a hydrophobic barrier that is sufficient to reduce the accessibility of sucrose to the taste buds. This proposes that the migration of sucrose molecules to the taste buds is not sufficiently reduced and consequently sweetness perception is not altered. An additional explanation might be due to the morphology of the tongue. Fungiform papillae, which contain taste buds with taste receptor cells (TRCs), are mainly found in the front part of the anterior tongue. The front part of the anterior tongue has consequently been demonstrated to be more sensitive for taste perception.9 In previous studies, it was found that less oil deposits at the front part of the anterior tongue compared to the back part (Pivk *et al.*, 2008b, Camacho *et al.*, 2014 and 2015). Taking the uneven distribution of oil deposition on the tongue into account, tastants could likely migrate to the taste buds and bind to the TRCs present at the tip of the tongue since the amount of oil deposited there is even smaller than on the back part of the tongue. Consequently, no effect of oral oil deposition on sweetness intensity is observed.

The results of this study did not confirm previous findings in literature (Lynch *et al.*,1993, Valentová and Pokorný, 1998, Ahn *et al.*, 2002) which reported that sweetness intensity was suppressed by preceding consumption of bulk oil or dispersed oil. Lynch *et al.*, (1993) found that the consumption of either coconut oil or sunflower oil reduced subsequent sweetness intensity of a sweet gelatin gels compared to preceding consumption of water. Valentová and Pokorný (1998) found that the consumption of bulk sunflower oil reduced subsequent sweetness intensity of sucrose solutions. Both studies used oils to coat the tongue, whereas this study used o/w emulsions with a maximum oil content of 20% (w/w). The difference in oil content between stimuli used in literature and our study led to differences in the amount of oil fraction deposited on the tongue. It is likely that the oil coatings formed with bulk oil form a hydrophobic layer and might have

created a thicker physical barrier and thus suppress the migration of hydrophilic tastants to the taste buds leading to a suppression of sweetness perception. Further, coconut oil seemed to have a higher effect in the suppression of sweetness intensity compared to sunflower oil (Lynch *et al.*, 1993). Coconut oil has a higher viscosity compared to sunflower oil (De Dios, 1995). Viscosity was found to affect taste intensity indicating a taste-texture interaction (Kokini 1987). Taste-taste interactions could have also contributed to the sweetness suppression, as coconut oil has an inherent coconut taste which could have caused sensory adaptation. Therefore, texture-taste or taste-taste interactions might have contributed to the suppression of sweetness intensity due to preceding coconut oil consumption found by Lynch *et al.*, (1993). Next to this, the sunflower oil used by Valentová and Pokorný (1998) was refined with oily and buttery flavors which might also have partly suppressed the subsequent perceived sweetness by flavor-taste interactions. Furthermore, although these studies used panels experienced with time-intensity and sensory profiling it is not explicit whether and how the panels were trained specifically on sweetness perception. The training of subjects to assess taste intensity and the resulting discrimination sensitivity of the subjects used is likely to differ between the different studies. Highly trained subjects should be used since the effect of oil coatings on subsequent taste perception are expected to be small.

Ahn *et al.*, (2002) and Valentová and Pokorný (1998) both observed sweetness intensity suppression after consumption of bulk oils or foods with high oil content. Those studies used sucrose solutions with a concentration of 0.12% (w/w) and 15.5% (w/w), respectively. In our study we did not observe sweetness intensity suppression. We used a sucrose concentration of 4.0% (w/w) and o/w emulsions with up to 20% (w/w) oil. This suggests that the fraction of oil deposited on the tongue is the main factor that determines whether suppression of taste intensity due to oral oil coatings occurs.

Valentová and Pokorný (1998) found that consumption of commercial emulsions, *i.e.* yogurt dressing, flavored emulsion and mayonnaise, resulted in a decrease on

subsequent sweetness intensity. However, these products have a complex food matrix and do not only contain fat, but also tastants and aromas. The ingredients and flavor might have contributed to the suppression of the sweetness by various mechanisms, *e.g.* texture-taste, taste-taste or flavour-taste interactions.

Milk is known to form a mouth coating (Frost *et al.*, 2001). This coating effect was thought to alter perception, since the consumption of milk was found to reduce subsequent perception of sweetness (Ahn *et al.*, 2002). The suppression of sweetness intensity by milk might be better explained by taste interactions. The sweetness of milk (Frost *et al.*, 2001) might have resulted in adaptation leading to a suppression in sweetness intensity of the tastant solution. Another reason might be that tastants or aroma molecules present in the oral cavity after the consumption of milk might have masked the succeeding sweetness of the tastant solution.

In conclusion, the dynamics of oral oil deposition is fast with the maximum oil fraction adhering to the tongue surface occurring within the natural drinking time of one sip of a beverage. Longer oral processing does not increase the oil fraction deposited on the tongue. Oil fraction deposited on the tongue after orally processing o/w emulsion with up to 20% (w/w) oil did not affect subsequent sweetness perception of sucrose solutions. We suggest that this is caused by a limited fraction of oil being deposited on the tip of the tongue where humans have a high density of taste papillae and consequently are most sensitive for taste perception. We suggest that the oil droplets deposited on the tongue did not form a hydrophobic barrier that is sufficient to reduce the accessibility of sucrose to the taste buds and consequently does not suppress taste perception.

Abbreviations Used:

Oil-in-water: o/w. Visual Analogue Scale: VAS.

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Formation, Clearance and Mouthfeel Perception of Oral Coatings formed by Emulsion-filled gels

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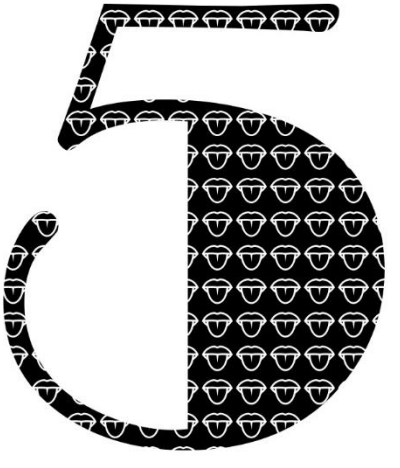

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Abstract

Four emulsion-filled gelatin gels varying in fat content (5 and 15%) and type of emulsifier (whey protein isolate: fat droplets bound to matrix; tween 20: fat droplets unbound to matrix) were studied. We investigated (i) the formation and clearance dynamics of fat deposition on the tongue using in vivo fluorescence during oral processing, (ii) influence of fat droplet characteristics on fat deposition on tongue and fatty mouthfeel, and (iii) effect of follow-up consumption (water or gelatin gel) on the removal of fat deposition on the tongue.

We conclude that fat fraction deposited on tongue and fatty perception increased with increasing mastication time, and decreased after expectoration with increasing clearance time. Fat fraction deposited on tongue and fatty perception are higher in gels with unbound droplets compared to bound droplets, as well as in gels with 15% fat compared to 5% fat. Water removed deposited fat from the tongue faster than gelatin gel.

Keywords: emulsion-filled gel, fat deposition on tongue, *in vivo* fluorescence, mouth-feel, oral coating, sensory perception

1. Introduction

Emulsion-filled gels are widely investigated as models for semi-solid and solid foods, such as yoghurts, cheeses, and processed meat products (Foegeding *et al.*, 2011; Dickinson, 2012). Using model gels allows to control structural, physical-chemical and mechanical properties of the foods in order to investigate the impact of specific properties on i.e. oral processing behavior and sensory perception. The structure and sensory perception of emulsion-filled gels during different phases of oral processing is known to be dynamic and multidimensional (Foegeding *et al.*, 2011; Stokes *et al.*, 2013; Liu *et al.*, 2015). During oral processing, emulsion-filled gels undergo continuous structure changes, including breakdown into smaller fragments, release and coalescence of fat droplets and formation of a cohesive bolus by saliva incorporation (Foegeding *et al.*, 2011; Stieger and van de Velde, 2013). In the first phases of oral processing (i.e. first bite and chewing), mechanical and rheological properties of emulsion-filled gels influence texture perception (Brandt *et al.*, 1963; Fischer and Windhab, 2011; Chen and Stokes, 2012). In the later phases of oral processing, the tribological and bolus properties influence texture perception (De Wijk *et al.*, 2006; Chen and Stokes, 2012; Liu *et al.*, 2015). After swallowing, food residues can remain adhered to oral surfaces to form an oral coating, which dominates the mouthfeel and after-feel perception (De Wijk *et al.*, 2009; Camacho *et al.*, 2014).

Previous studies have suggested that the release of fat from emulsion-filled gels and coalescence of fat droplets under mouth-mimicking *in vitro* conditions is related to a decrease in friction, leading to an increase in perception of fat-related sensory attributes (Dresselhuis *et al.*, 2007; Sala *et al.*, 2007b; Liu *et al.*, 2015). Evidence for an increase of the amount of fat deposited in the oral cavity during oral breakdown of emulsion-filled gels due to increased fat release and coalescence is not available. The oil fraction deposited on the tongue after consuming oil or o/w emulsions was quantified using an *in vivo* fluorescence methodology (Pivk *et al.*, 2008; Camacho *et al.*, 2014). They reported that oil fraction deposited on the

tongue and fat-related mouthfeel and after-feel perception increased with increasing oil content in liquid o/w emulsions. Knowledge on the behavior and after-feel perception of oral coatings formed by liquids has increased in the last years. However, studies describing oral coatings formed by semi-solid and solid foods are scarce. To our knowledge, the study of Repoux *et al.*, (2012) was the first and only one that investigated the formation of oral fat coatings formed by solid foods (cheeses) (Repoux *et al.*, 2012). In the mentioned study, the oral coatings were collected by asking the participants to rinse their mouth with water after masticating cheeses. The oral coatings were then quantified *ex vivo* in the rinsed water using fluorescence spectroscopy. Although this method is fast and easy to apply, it might have the limitation that the rinsing of the coating is incomplete, so that some of the fat in the oral coating remains attached to the oral surface and is not quantified. Therefore, it is desirable to quantify oral coatings directly in mouth by *in vivo* fluorescence spectroscopy without an extraction/collection step.

Since the oral processing is dynamic, the formation and clearance of the oral coatings is also dynamic. Due to the movement of tongue against teeth and palate the formation of the oral coatings can be disrupted and the clearance can be enhanced (De Wijk *et al.*, 2009). Secretion of saliva is also known as a factor contributing to the clearance of coatings. For instance, the formation of the lubricating saliva film (Carpenter, 2012), enzyme activity (Carpenter, 2012), as well as the salivary flow over oral surfaces (Sas and Dawes, 1997; Adams *et al.*, 2007) can improve the clearance of oral coatings formed by food residues. Similarly, drinking liquid foods and/or chewing solid foods, would introduce intensive mechanical disruption of the oral coatings due to the flow of liquids and the existence of solid food particles.

The aim of this study was to determine the influence of oral processing and fat droplet characteristics of emulsion-filled gels on the formation and clearance of fat deposition on the tongue in relation to mouthfeel and after-feel sensory perception. We investigated (i) the fat fraction deposited on the tongue during the formation and clearance of oral coatings, (ii) the influence of fat droplet

characteristics on fat fraction deposited on the tongue and dynamic sensory perception, and (iii) the effect of follow-up consumption of beverages and foods (water or gelatin gel) on the clearance dynamics of fat deposited on the tongue. We selected four model emulsion-filled gels varying in fat content (5 and 15%) and emulsifier type (fat droplets either bound to or unbound from matrix). These emulsion-filled gels were characterized by their rheological, tribological and microstructural properties. The formation and clearance of fat deposited on the tongue was determined by *in vivo* fluorescence. Sensory perception of the gels was quantified during and after oral processing.

We hypothesize that:

1a) Fat fraction deposited on the tongue and fatty mouthfeel perception increase during formation of oral coatings with increasing oral processing time;

1b) Fat fraction deposited on the tongue and fatty mouthfeel perception decrease during clearance of oral coating after expectoration of emulsion-filled gels;

2) Emulsion-filled gels with higher fat content and unbound fat droplets have higher fat fraction deposited on the tongue and higher intensity of fatty mouthfeel perception;

3) Consumption of solid foods (gelatin gel) leads to faster clearance dynamics of fat deposition on the tongue compared to consumption of liquids (water) due to mechanical abrasion of the coating.

2. Materials and Methods

2.1 Materials

Soft pork fat was kindly provided from Ten Kate Vetten B.V. (Ter Apelkanaal, The Netherlands). Porcine skin gelatin (bloom value 240-260) was kindly provided by Rousselot (Gent, Belgium). Powdered Whey Protein Isolate (WPI, Bipro™) was purchased from Davisco International Inc. (La Sueur, MN, USA). Tween 20 (Polyoxyethylene sorbitan monolaurate) and paraffin oil were

purchased from Sigma-Aldrich (St. Louis, MO, USA). Sweetener (Natrena, main components: cyclamate, saccharin and acesulfame-K), vanilla flavor (Dr. Oetker) were bought from local supermarkets (Wageningen, the Netherlands). Curcumin (7% Curcumin solution in propylene glycol and polysorbate, L-WS) was obtained from Sensient (the Netherlands). Pig's tongues were kindly donated by the VION Food Group (the Netherlands). All materials were used without further purification. All samples for sensory evaluation were prepared under food grade conditions. All samples were prepared with regular tap water.

2.2 Sample Preparation

Compositions of the emulsion-filled gels are given in **table 5.1**. The preparation of emulsion-filled gels was described in detail previously (Liu *et al.*, 2015). First, two 40% (w/w) o/w emulsions stabilized with either 1% (w/w) WPI or 2% (w/w) Tween 20 were prepared. The homogenization pressure was 310 bar for the WPI stabilized emulsions and 180 bar for the Tween 20 stabilized emulsions, yielding fat droplets with average droplet size of $d_{3,2}$ = 1.5 ± 0.3 μm. The droplet size distributions of both emulsions were measured with a MasterSizer200 (Malvern Instruments Ltd., Malvern, UK). The calculation of droplet size distribution was based on a uni-model with fitting accuracy above 99%. Curcumin was added to the emulsions (450 μl of the 7% stock solution per 100g emulsion) for fluorescence quantification of fat content.

The emulsions were then mixed with gelatin solutions. Sweetener and vanilla flavor were added to the gel solutions to enhance the palatability of the emulsion-filled gels, and to mask the bitter taste of Tween 20 and the pork flavor of pork fat. The concentrations of sweetener and vanilla flavor were determined by achieving similar taste and flavor intensity of the gels. The gel mixtures were immediately stored in the refrigerator at 5 °C for 20 h and then kept at 20 °C for at least 2 h prior to the sensory test and characterization.

Table 5.1. Composition of the emulsion-filled gels

Emulsifier (w/w, in water phase)	Fat content (% w/w)	Gelatine (% w/w)	Sweetener (% w/w)	Vanilla flavor (% w/w)
1% WPI	5	4	1.43	0.93
	15	4	1.28	2.78
2% Tween 20	5	4	1.90	0.93
	15	4	2.13	2.78

The emulsion-filled gels used for the oral processing studies were allowed to gel in 30 mL plastic syringes (internal diameter 21.3 mm) coated with a thin layer of sunflower oil. Gels for tribological measurements, microstructure analysis and fat release measurement were prepared in 30 mL plastic syringes without coating oil layer. Gels for large deformation measurements were allowed to gel in 60 mL plastic syringes (internal diameter 26.4 mm) coated with a thin of layer paraffin oil.

2.3 Characterization of Emulsion-filled gels

Large deformation properties and microstructural analysis were carried out as described by Liu *et. al.*. (Liu *et al.*, 2015). An Instron universal testing machine (M5543, Instron International Ltd., Belgium) equipped with plate–plate geometry was used to perform uni-axial compression tests on emulsion-filled gels. The cylindrical gel specimen was 25 mm high, and the diameter of gel specimen was 26.4 mm. To determine Young's modulus, fracture stress and fracture strain, all measurements were performed at a constant compression speed of 1 mm/s up to a compression strain of 80%. To determine recoverable energy, the measurements were performed at a constant compression and decompression speed of 1 mm/s up to a compression strain of 20%. Microstructural analysis of emulsion-filled gels were stained with 0.5% (w/w) Nile blue solution to visualize the fat phase. CLSM images were recorded on a LEICA TCS SP5 Confocal Laser Scanning microscope

(Leica Microsystems CMS GmbH, Manheim, Germany) equipped with an inverted microscope (Leica DM IRBE). The Argon laser and HeNe633 laser were used.

Friction force of the emulsion-filled gels was measured with a tribometer based on the method by Liu et. al. The friction force of the small piece of intact gel was the same as the pre-treated gel (data not shown). For the convenience of measurement, instead of a pre-treated gel, an intact piece of gel (about 200 mg) was sheared in the tribometer between a tribo-pair consisting of a glass plate and a PDMS probe. The load was set to 0.5 N. During each measurement the glass plate was oscillating at the speed 80 mm/s (Dresselhuis *et al.*, 2007). Friction force was determined during the shear movement. For each measurement a new probe was used and the glass surface was cleaned with water and ethanol. All the measurements were conducted at 20 °C.

2.4 Quantification of in vitro Fat Release

To quantify the *in vitro* fat release from semi-solid emulsion-filled gels after shearing, the method developed by (Sala *et al.*, 2007b) was used. Emulsion-filled gels were squeezed out through the small orifice (d = 0.9 mm) of a syringe by applying on the plunger a constant velocity (10 mm/s) with a Texture Analyzer (TA, Stable Micro Systems). A known amount of sheared gel (typically 15 g) was collected in a centrifuge tube and subsequently diluted by typically 30 g of demi water (to reach a 1:2 dilution ratio). The diluted sheared gel was gently vortexed for 15 s and then centrifuged at 3000 g for 5 min. The supernatant were filtered using Acrodisc syringe filters (5 μm pore size, PALL Corporation) (Devezeaux de Lavergne *et al.*, 2015). The filters should allow the fat droplets that were released into the water phase to pass through the pore, since the fat droplet size was smaller than pore size. The fat content of the filtrate was quantified with the Röse-Gottlieb method (ISO 1211) by Qlip (Zutphen, The Netherlands). The fat release measurements per gel variant were conducted in triplicate. The

quantification of fat content per filtrate was performed in duplicate. The measurements of fat droplet release were conducted at 20 and 37 °C.

2.5 *In vivo* Determination of Fat Fraction Deposited on the Tongue Surface

In vivo Fluorescence Measurements

The method used in this study was previously described in detail (Camacho *et al.*, 2014). Fluorescence measurements were made with a single point measurement (Fluorolog Instruments SA Inc, Jobin Yvon Spex) at an excitation wavelength of 440 nm, an emission wavelength of 495 nm with a slit width of 0.95 mm and a measurement time of 0.1 s. To measure the fluorescence intensity on the tongue surfaces, fluorescence remote read fiber optic probe was used. To ensure the the distance between the probe and the surface remain constant, a plastic ring (diameter of 16.9 mm and height of 5 mm) was attached to the end of the probe. The probe was put gently on the front part of the anterior tongue during the measurements. To convert the fluorescence intensity measured on the subjects tongue surface to the fat fraction deposited on the tongue surface, *i.e.* mass of fat per area of tongue (mg/cm^2), calibration lines were made following the procedure previously described (Camacho *et al.*, 2014). In short, calibrations were made with pieces of the middle part of the pig's tongue at 37.5 ^{o}C. The emulsion-filled gels were heated in a water bath, until they reached 37 ^{o}C and were completely melted. The emulsion-filled gels were then kept at room temperature and spread on the surface of the pig's tongue (2 cm x 2 cm). The fluorescence intensity of the pig's tongue with the melted gel was measured. A fresh piece of tongue was used for each measurement. As the ingredients of each gel could affect the fluorescence intensity of curcumin, a calibration curve (fluorescent intensities *vs.* fat fraction on tongue surface) was made for each of the four gels. All calibration measurements were performed in triplicate and are shown in the supporting information.

Selection of the Subjects

Twenty-five subjects participated in the screening session (10 males and 15 females; mean age of 26 ± 2.8 years). Exclusion criteria were smokers, braces, tongue piercings, vegetarians or applicable allergies. Participants gave informed written consent and received a financial compensation for their participation. The study did not require ethical approval by the local medical ethics committee under Dutch regulations. The study was conducted in line with the declaration of Helsinki.

Mastication behavior can vary between subjects and influence the fat fraction on the tongue and the perception of fat. In order to perform the measurements with a homogeneous panel, a pre-selection of subjects was conducted based on: (i) natural mastication time of the gel and, (ii) understanding of the attributes. The subjects were asked to measure the mastication time for two 5 mL emulsion-filled gels (gels with 15% bound droplets and gels with 15% unbound droplets – gels with the highest and lowest Young's modulus) until the natural intention to swallow the sample. Each emulsion-filled gel had a three-digit code and was presented to the subject in triplicate in random order. Subjects with a mastication time with a |z-value| ≥2 were excluded from the study. The average mastication time for the gels of the selected group was approximately 8 s (7.7 ± 0.7 s). The selected subjects were invited for an introduction session during which the definition of the sensory attribute used in the study "fatty" and the evaluation protocol were explained (**table 5.2**).

Table 5.2. Definition of the sensory attribute and evaluation protocol

Sensory attribute	Definition	Evaluation protocol
Fatty	Sensation of feeling a layer of fat covering the mouth after the food is spat out	Slide the tongue on the palate and lips and the lips on one another

The four emulsion-filed gels were presented in different pairs to the subject. The perception of the attribute was discussed. Thirteen subjects were selected to participate in the study (6 males and 7 females; mean age of 26 ± 2.9 years).

Determination of Fat Fraction Deposited on Tongue Surface

The formation and clearance of the fat fraction deposited on the anterior back part of the tongue was determined with *in vivo* fluorescence spectroscopy. In order to study the fat coating formation, 5 mL of each emulsion-filled gel (room temperature) was processed in mouth for either 33% (2 s), 66% (5 s) or 100% (8 s) of mastication time. The 100% mastication time of the gels was determined to be approximately 8 s (7.7 ± 0.7 s) during the screening session. After processing the gel in mouth for a specific time, the gel was spat out and the fluorescence was measured on the anterior back part of the tongue. When the gel was processed in mouth for 8 s, the clearance of the coating was measured at 15, 30, 45, 60, 90, 120 and 180 s after expectoration of the emulsion-filled gel. The subjects were not allowed to speak or drink during the measurements. After the measurement, subjects cleaned their tongue with water, crackers and a tongue scraper. Each gel was tested in triplicate. Samples had a three-digit code and were completely randomized over the sessions and per subject. Each session of 60 min consisted of the measurements of 6 samples. Each gel x time was measured in triplicate.

Determination of Mouthfeel and After-feel Perception of Oral Coatings

During the fluorescence measurements, subjects performed sensory evaluation of the mouthfeel attribute fatty at 33, 66 and 100% of mastication time. Evaluation of the intensity of the sensory attribute was made on a 100 mm VAS line scale as used in the sensory analysis during the fluorescence measurements. The VAS line scale had anchors "little" and "very" at the 5% edge of the line. After the emulsion-filled gel was processed in mouth for 8 s, it was expectorated. The intensity of the after-feel attribute fatty was evaluated at 30, 60, 90, 120 and 180 s after expectorating gel. All 13 subjects had no training further than the introduction session.

Determination of Fat Clearance due to Follow-up Consumption

To investigate the effect of follow-up consumption on the removal of fat coatings, the same panel as described before (13 subjects with mean age of 26 ± 2.9 years) participated in the study. Each set of measurements consisted of: (i) the formation of a fat coating by a liquid emulsion and (ii) oral processing of a possible fat removal agent: water or gelatin gel for different times. The fat fraction was determined with *in vivo* fluorescence spectroscopy on: (i) after the formation of the fat coating by the emulsion and (ii) after the oral processing of water or gelatin gel.

A 15% (w/w) emulsion was used to form the fat coating in mouth. This 15% (w/w) emulsion was diluted from a 40% fat emulsion stabilized with 2% Tween as described before. This 15% (w/w) emulsion contained 1% (w/w, water phase) sweetener and 2.78% (w/w) vanilla flavor. Further, 4% gelatin gels were prepared as described in the previous section (Sample preparation).

A sip (20 mL) of 15% (w/w) fat emulsion (room temperature) was processed in mouth for 30s. After expectoration of the emulsion, fluorescence was measured on the anterior back part of the tongue (t=0s). Immediately after, a 5 gram of 4% gelatin gel or 5 mL of water was processed in mouth for either 2, 5 or 8s. Afterwards, the gelatin gel or water was spat out and the fluorescence was measured. Each set of measurements consisting of emulsion + (water or gelatin gel) x oral processing time was measured in triplicate.

2.6 Statistical Data Analysis

SPSS® Statistics version 21 was used for the statistical data analysis of results obtained from sample characterization. Tukey's test was performed as a post-hoc test where applicable. Data were tested on significant differences among the four types of emulsion-filled gels. Level of significance was set at $p<0.05$.

SPSS® Statistics version 19 was used for the statistical data analysis of the results obtained from the sensory and *in vivo* fluorescence measurements. Descriptive statistics were used to obtain the mean and standard error (SE). Outliers ($z>2$)

were removed from the data. Fluorescence intensity data of both formation and clearance of the coating and data for mechanical clearance was normalized with a square root transformation. The effect of gel (within subject factor; 5% unbound, 15% unbound, 5% bound and 15% unbound), time (within subject factor; 0, 15, 30, 45, 60, 90, 120, 180s after expectoration or within subject factor: 2, 5 and 8s mastication time) and interactions on fat fraction deposited on the tongue were tested by repeated-measures ANOVA. For the sensory data, a repeated-measures ANOVA was used to investigate the effect of gel (within subject factor; 5% unbound, 15% unbound, 5% bound and 15% unbound) and time (within subject factor; 0, 15, 30, 45, 60, 90, 120, 180s after expectoration or within subject factor: 2, 5 and 8s mastication time) and interactions on fatty film intensity. The effect of follow-up consumption (water and gelatin gel) and processing time (0, 2, 5 and 8s) and interactions on fat fraction deposited on tongue were tested by repeated-measures ANOVA. A significance level of $p<0.05$ was chosen. The degrees of freedom for all the effects which were shown by Mauchly´s test to violate the assumption of sphericity were corrected using Greenhouse-Geisser estimates. Pairwise comparisons using Bonferroni tests were analyzed in case the effects were significant.

3. Results

3.1 Characteristics of Emulsion-filled gels

The mechanical properties and microstructure of emulsion-filled gels containing curcumin, flavor and sweetener were characterized. This allows us to check the impact of addition of these ingredients on the properties of emulsion-filled gels compared to plain emulsion-filled gels used in previous studies.

Large Deformation Properties

Figure 5.1 A shows that with increasing fat content from 5 to 15%, the Young's modulus of the emulsion-filled gels increases when fat droplets are stabilized with WPI, while decreases when fat droplets are stabilized with Tween 20. Young's

moduli of gels with droplets stabilized with Tween 20 are lower than those stabilized with WPI.

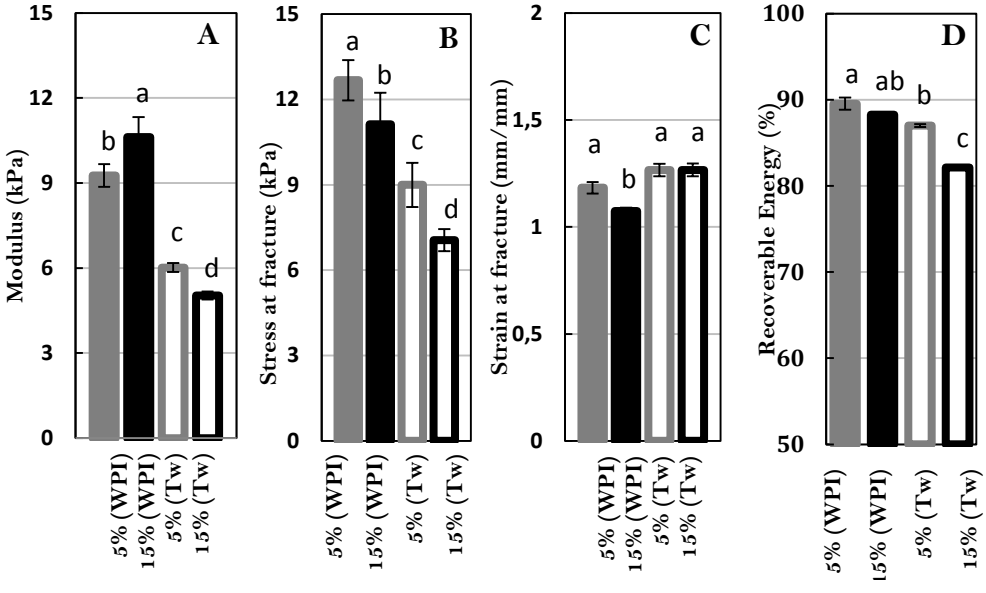

Figure 5.1. Large deformation properties of emulsion-filled gelatin gels. **A**: Young's modulus; **B**: Stress at fracture; **C**: Strain at fracture. **D**: Recoverable energy. In each sub-figure: Grey color=5% fat; Black color=15% fat; filled bars = droplets stabilized with 2% Tween 20. Error bars represent standard deviation.

Figure 5.1 B shows that for both gels with bound droplets and unbound droplets, fracture stress decreases significantly ($p<0.05$) with increasing fat content. The fracture stress of gels with bound droplets is larger than those with unbound droplets. **Figure 5.1 C** shows a slight but significant decrease in fracture strain of gels with bound droplets as fat content increases ($p<0.01$), while the fracture strain of gels with unbound droplets is not significantly influenced by varying fat content from 5% to 15%. **Figure 5.1 D** shows that the recoverable energy of emulsion-filled gels containing bound droplets is not significantly decreased with increasing fat content, while a significant decrease is observed when droplets are unbound from the gel matrix ($p<0.001$).

Microstructure

Figure 5.2. shows the microstructure of the four emulsion-filled gels. Droplets that are stabilized with 1% WPI (bound) are more homogenous distributed in the gel matrices than droplets that are stabilized with Tween 20 (unbound). Droplet aggregation is observed to a limited extent, but no coalescence of droplets is observed.

5% fat		**15% fat**	
Bound	Unbound	Bound	Unbound

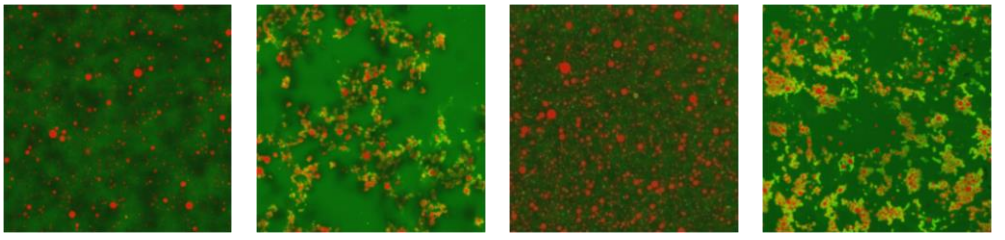

Figure 5.2. CLSM images of fat emulsion-filled gels. Red phase represents fat, green phase represents gelatin matrices. Image size 160mm x 160 mm.

Tribological Properties

Figure 5.3 shows the friction force measured for an intact piece of gel sheared between two tribo-surfaces at 80 mm/s. For gels with bound droplets, increasing fat content from 5 to 15% leads to a significant decrease in friction force ($p<0.001$). For gels with unbound droplets, increasing fat content also leads to a significant decrease in friction force ($p<0.05$), but to a lesser extent than for gels with bound droplets. For gels with lower fat content (5%), the gel with unbound droplets has significantly lower friction force than the gel with bound droplets ($p<0.001$). For gels with higher fat content (15%), the gel with unbound droplets has a friction force that is not significantly different from the gel with bound droplets.

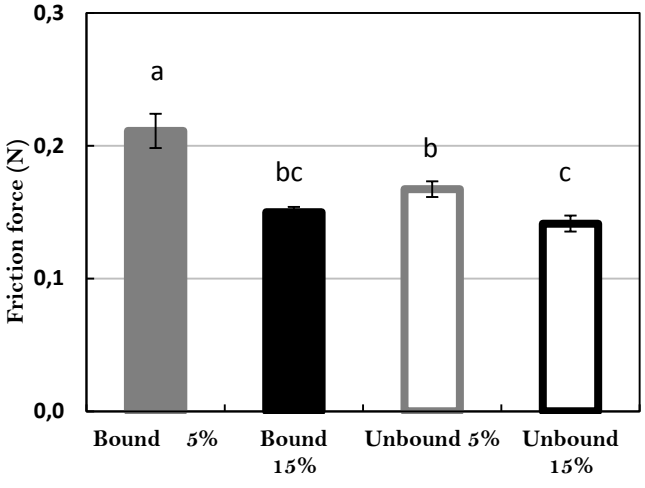

Figure 5.3. Friction force of emulsion-filled gels measured at shearing speed of 80 mm/s. Error bars represent standard deviation.

In vitro Fat Droplet Release

The fat droplets were extracted from emulsion-filled gels at 20 and 37 °C. **Figure 5.4** shows that at 20 °C, less than 0.4% fat was released from the sheared gels with 5% bound droplets, and less than 1% fat was released from the sheared gels with 15% bound droplets. For gels with 15% unbound droplets, significantly more amounts of fat were released from the gel matrices than gels with 15% bound droplets ($p<0.001$). The amount of fat released is proportional to the original fat content of the emulsion-filled gel. At 37 °C, the fat droplet release for both unbound and bound droplets is the same and is proportional to the fat content of the emulsion-filled gel.

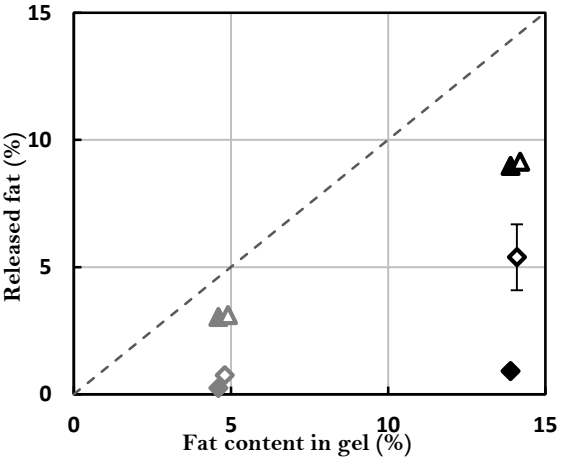

Figure 5.4. In vitro fat droplet release as a function of fat content in emulsion-filled gels. Diamond symbols represent 20°C; triangle symbols represent 37°C. Filled symbols represent bound droplets; empty symbols represent unbound droplets (slightly shifter to the right to avoid overlapping with other points). Error bars represent standard deviation. Dashed line indicates total oil droplet release

3.2 Formation of Fat Deposition on Tongue during Oral Processing and Mouthfeel Perception

Figure 5.5 A depicts the effect of oral processing time of the emulsion-filled gels on the formation of fat deposits on the back part of the anterior tongue. A significant main effect of sample [$F(2.5, 96.5)=160.1$, $p<0.001$] and time [$F(2, 76)=3.2$, $p<0.05$] on fat fraction was observed. No interaction effect of sample x time ($p=0.653$) was found on fat fraction.

The longer the gels were processed in mouth the higher the fat fraction deposited on the tongue. This trend is clearer for the 15% fat emulsion-filled gels. The coatings formed by the gels with 15% fat created a significant higher fat fraction ($p<0.001$) than the coatings formed by the gels with 5% fat at any oral processing time. The gels with unbound droplets created a higher fat fraction deposited on the tongue than the gels with bound droplets. This trend was significant for the gels with 5% fat at 2 s (33% mastication time) and at 8 s (100% mastication time), $p<0.05$.

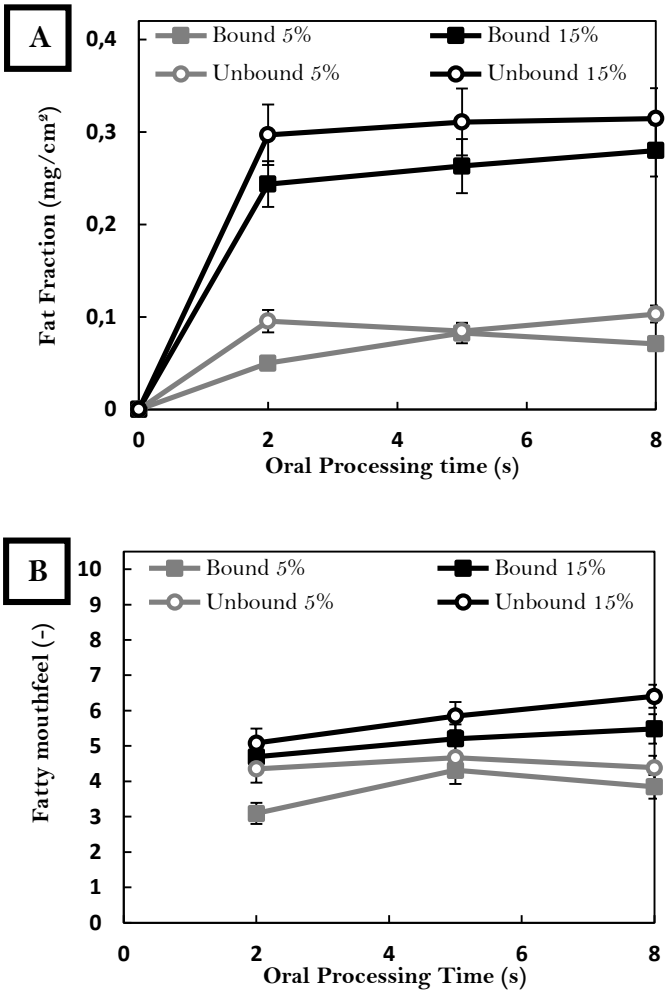

Figure 5.5. A. Fat fraction deposited on the back of the anterior part of the tongue as a function of oral processing time (s) of emulsion-filled gels. **B**. Fatty mouthfeel perception as a function of oral processing time (s) of emulsion-filled gels. Each data point represents the average of n=13 subjects and 3 replicates. Lines are drwan to guide the eye. Error bars represent standard error.

Figure 5.5 B shows the effect of oral processing time of the gels on the mouthfeel perception of the attribute fatty. Sample [$F(3, 114)=27.0$, $p<0.001$] and time [$F(2, 76)=7.9$, $p=0.001$] had a significant main effect on the perception of fatty mouthfeel of the coating. No interaction effect of sample x time ($p=0.121$) on the perception of fatty mouthfeel of the coating was found. The trend observed for the

perception of fatty mouthfeel is similar to the trend of fat deposition on the tongue. The longer the gels were processed in mouth the higher the fatty mouthfeel perception. This trend was significant for the gel with unbound droplets and 15% fat from time 2s to time 5s and 8s ($p<0.05$). The coatings formed by gels with 15% fat were perceived as more fatty than the coatings formed by gels with 5% fat, although the trend is not significant. It is interesting to note that the gel with 5% fat and unbound droplets was not perceived significantly different from the gel with 15% fat and bound droplets ($p=0.105$). Nevertheless, when comparing gels with the same interaction oil droplet/matrix and different fat contents, results show that gels with 15% fat and bound oil droplets had a significant higher fatty mouthfeel perception than gels with 5% fat, for the three processing times ($p<0.001$). Further, gels with 15% fat and unbound oil droplets had a significant higher fatty mouthfeel perception compared to gels with 5% fat at oral processing times of 5 seconds and 8 seconds ($p<0.05$).

3.3 Clearance of Fat Deposition on Tongue and After-feel Perception

Figure 5.6 A shows the clearance of fat fraction deposited on the tongue as function of time after expectoration of the gels. For these measurements, each gel was processed in mouth for 8 s (100% natural oral processing time) and then expectorated. Afterwards, the clearance of the fat coating was analyzed at different time points. A significant main effect of sample [$F(3, 114)=52.7$, $p<0.001$], time [$F(2.6, 99.1)=204.8$, $p<0.001$] and sample x time [$F(7.4, 281.2)=20.2$ $p<0.001$], on fat fraction was observed. Until 30 s after expectoration, the fat fraction of gels with 15% fat remained significantly higher compared to gels with 5% fat ($p<0.05$).

Figure 5.6 B shows the fatty after-feel perception as a function of time after the gel was expectorated. Results show a significant main effect of sample [$F(3, 114)=18.3$, $p<0.001$], time [$F(1.7, 64.3)=243.2$, $p<0.001$] and sample x time [$F(7.6, 287.8)=6.1$, $p<0.001$] on the fatty after-feel perception. Following the same trend as in the fat fraction clearance, the coatings formed by gels with 15%

fat were perceived as significantly more fatty compared to the coatings formed by gels with 5% fat up to time 30 s after expectoration.

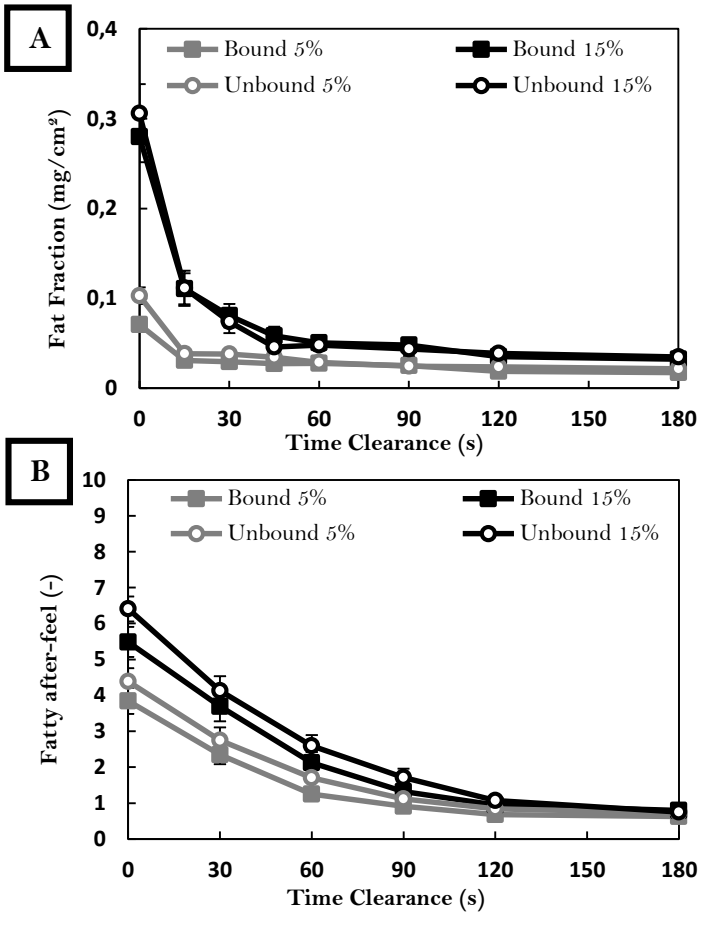

Figure 5.6. A. Fat fraction deposited on the back of the anterior part of the tongue as a function of time (s) after each emulsion-filled gel was expectorated. **B**. Fatty mouthfeel perception as a function of time (s) after each emulsion-filled gel was expectorated. Each data point represents the average of n=13 subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent standard error.

3.4 Clearance of Fat Coating due to Follow-Up Consumption

Figure 5.7 depicts the effect of different processing time of plain water or plain gelatin gel on the clearance dynamics of fat fraction deposited on the tongue.

Significant main effect of water and gelatin [F(1, 38)=52.6, p<0.001], time [F(2.2, 82.2)=80.1, p<0.001] and water and gelatin x time [F(2.4, 91.7)=6.72, p=0.001], on fat fraction was observed. Clearance of fat fraction using water was more effective at every time point tested (p<0.001) compared to using gelatin gels. Longer oral processing time of both water and gelatin gel lead to a lower fat fraction (significantly decrease from 0 s till 2 s).

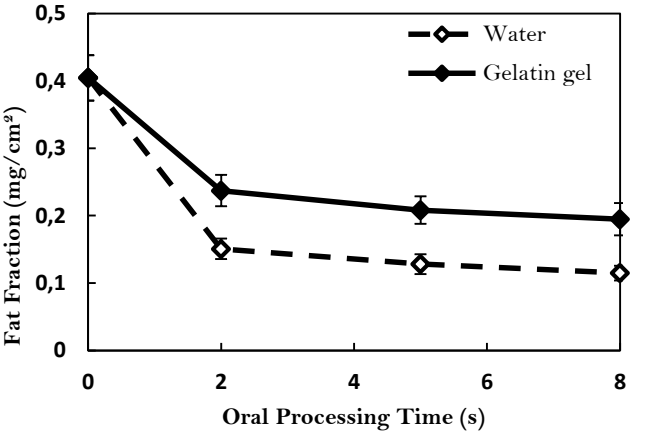

Figure 5.7. Fat fraction deposited on the back of the anterior part of the tongue as a function of processing time of water and gelatin gel. Time 0s corresponds to the fat fraction on the tongue after a 15% (w/w) fat emulsion stabilized with Tween 20 was processed in mouth for 30s and expectorated. Each data point represents the average of n=13 subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent standard error.

4. Discussion

4.1 Characteristics of Emulsion-filled Gels

The Young's modulus of gels increased with increasing fat content as droplets are stabilized with WPI, while decreased as droplets are stabilized with Tween 20. From this we confirm that droplets that are stabilized with WPI are bound to the gelatin matrix, and droplets that are stabilized with Tween 20 are not bound to the matrix. This agrees with previous findings reported in literature. The results of fracture stress, fracture strain, and recoverable energy are also comparable to data reported for plain emulsion-filled gels (Sala *et al.*, 2007; Liu *et al.*, 2015).

During the preparation of the Tween 20 stabilized emulsion-filled gels, we observed slight degree of reversible phase separation, which is probably due to depletion interactions between droplets and gelatin. This might explain why slight droplet aggregations are observed for the gels with unbound droplets. The microstructures of these gels are comparable to previous studies (Liu *et al.*, 2015).

At 20 °C, the release of the bound fat droplets from the gel matrices was very limited, because most probably droplets were inside and bound to the broken gelatin gel pieces. The unbound fat droplets were released significantly more from the matrix. This is in accordance with literature (Sala *et al.*, 2007b; Liu *et al.*, 2015). At 37 °C the gelatin was completely melted, therefore bound droplets also became "unbound". This explains why the release of droplets for both types of gels at 37 °C was the same. In none of the samples we observed a 100% droplet release. This is probably because the pore size (5 μm) of the filter that we used for fat extraction is not big enough. In any case, our data strongly confirms our hypothesis that gels with unbound droplets released more fat at 20 °C after shearing than gels with bound droplets, and same amount of fat when melted.

Since gels containing unbound droplets could release more fat at 20 °C, we expected the friction force of gels with unbound droplets would be lower than gels with bound droplets. We observed in the tribological results that at the same fat content, gels with unbound droplets had lower friction force than with bound droplets. This agrees with previous studies (Liu *et al.*, 2015).

To summarize, the addition of curcumin, flavor and sweetener to the gel does not considerable influence their mechanical properties and microstructures.

4.2 Formation of Fat Deposition on the Tongue and Mouthfeel Perception

Emulsion-filled gels with 15% fat formed fat depositions on the tongue which contained about 3 folds more fat than the fat deposition formed by gels with 5% fat (after 100% mastication time (8 s)). This is in accordance with previous studies (Camacho *et al.*, 2014) which demonstrated that with increasing fat content of

liquid stimuli processed in mouth the fat fraction deposited on the tongue increases.

Figure 5.5.A reveals that most of the fat is deposited on the tongue during the first 2 s of oral processing. This suggests that the first bites are the most relevant for the formation of fat depositions on the tongue. Further, fat fraction deposited on the tongue increased when oral processing time of the gels increased. This trend was clearer for gels with higher fat content (15%) compared to gels with lower fat content (5%). Further, fat droplets unbound to the matrix created higher fat deposition compared to fat droplets bound to the matrix. This is in line with the results from the *in vitro* fat release measurements at 20 °C. The difference in fat fractions between gels with unbound and bound droplets, however, is smaller than their difference in fat release at 20 °C. This is probably due to the melting of the gelatin matrix in mouth. The actual gel temperature in mouth is dynamic during oral processing, and it should be between 20 and 37 °C. Therefore, the fat fraction on the tongue surface should correspond more accurately to the *in vitro* fat release at a temperature between 20 and 37 °C.

The fatty mouthfeel perception followed the same trend as the fat fraction deposition on the tongue. In general, coatings with higher fat fractions led to higher intensities of fatty mouthfeel perception suggesting that the physical fraction of fat deposited on the tongue is sensed. Longer oral processing times led to more intense fatty mouthfeel perception. Likewise, fat droplets unbound from the matrix led to more intense fatty mouthfeel perception compared to fat droplets bound to the matrix. This is in accordance with previous research where a trained QDA panel evaluated comparable emulsion-filled gels (Liu *et al.*, 2015). This is also in accordance with the friction results (**Figure 5.3**). Gels that had lower friction were perceived more fatty. To summarize, differences in intensities of fatty mouthfeel are mainly due to the differences in fat deposition on the tongue and the consequent differences in friction forces.

4.3 Clearance of Fat Deposition on the Tongue and After-feel Perception

Fat fraction deposited on the tongue decreased with increasing time after the expectoration of the emulsion-filled gels. Clearance of fat depositions on the tongue is likely due to three main effects: saliva flow (Adams *et al.*, 2007), movements of the tongue against the palate, which can mechanically remove the fat from the tongue surface (Camacho *et al.*, 2014), and food particles that can remove the fat deposition on the tongue due to mechanical abrasion.

Fat fraction had the steepest decrease on the first 15 s after expectoration of the gels. This suggests that the first seconds after food consumption are the most relevant for the clearance of fat from the tongue surface. Up to 30 s after expectoration, the fat fraction from gels with 15% fat remained significantly higher compared to gels with 5% fat. The clearance of fat coatings formed by the emulsion-filled gels has a similar behavior to the clearance of fat coatings formed by oil-in-water (o/w) emulsions. Previous research showed that the oil fraction deposited on the tongue from o/w emulsions with 15% oil remained higher compared to o/w emulsions with 5% oil up to 30 s after expectoration of the sample (Camacho *et al.*, 2014). This similarity is likely due to the melting in mouth of the emulsion-filled gel at 100% mastication time. The melting of the gelatin matrix can lead to the emulsion-filled gel to behave like a high-viscous liquid o/w emulsion. When the melted gel is expectorated, the remaining fat deposited on the tongue is thus behaving comparable to fat deposited on the tongue formed by a liquid o/w emulsion.

Fatty after-feel perception of the emulsion-filled gels followed the same trend as the fat fraction clearance. Up to 30 s after expectoration, the fatty after-feel perception of gels with 15% fat remained significantly higher compared to gels with 5% fat. Nevertheless, in contrast to the fat fraction clearance, there was no steep decrease on the fatty after-feel perception, but rather a smooth decrease. This is likely due to adaptation effects. Adaptation is a decrease in responsiveness

under a constant stimulus (Lawless and Heymann, 2010). As the taste and mechanoreceptors have been continuously stimulated with the fat deposited on the tongue (throughout the mastication of emulsion-filled gels and after expectoration), it is possible that the subjects were less sensitive to be able to efficiently detect changes in the fat clearance from the tongue.

4.4 Clearance of Fat Coating due to Follow-up Consumption

Figure 5.7 shows that water flow has a stronger effect on the removal of the fat deposited on the tongue than masticating a gelatin gel. Higher saliva flow was shown to lead to faster oral coatings' clearance compared to low saliva flow (Adams *et al.*, 2007). It is possible that the effect of water flow in-mouth is more effective compared to the effect of the mastication of the gel, which is likely to remove the fat deposited on the tongue due to abrasion by the pieces of gelatin during mastication. Nevertheless, at longer oral processing times, the gelatin gel melts in mouth and likely forms a melted gelatin layer. The gelatin layer might protect the fat deposited on the tongue from removal caused by, for instance, the mechanical rubbing of the tongue against the palate. Although water is more effective removing the fat deposited on the tongue, the fat is still not completely removed. Thus, studies which rely on the rinsing method to quantify coatings, *i.e.* removal of coating by rinsing with water, probably underestimate the fat content in the oral coating by 27.5 - 37.5 % due to incomplete removal.

Figure 5.7 shows a steep decrease of fat fraction deposited on the tongue after the gelatin gel and water were processed in mouth for 2 s (around 50% fat decrease). This suggests once more, that the first seconds after food consumption are the most relevant for the fat clearance either with or without the effect of follow-up consumption (**Figure 5.6**). Further, the oral processing time of water and gelatin gel did not create a significant effect on the fat coating removal after 2 s, indicating that the main cause of fat coating removal is the different in-mouth behavior of the water and gelatin gel.

5. Conclusions

We conclude that fat fraction deposited on the tongue and fatty perception increase with increasing mastication time, and decrease after expectoration with increasing clearance time. Formation and clearance dynamics of the fat deposited on the tongue are fast processes. Fat fraction deposited on the tongue and fatty perception are higher in gels with unbound droplets compared to bound droplets, as well as in gels with 15% fat compared to 5% fat. Drinking water has a stronger effect on clearing the fat fraction from the tongue compared to chewing gelatin gel. We conclude that fat droplet characteristics, oral processing time, as well as follow-up consumption affect the amount of fat deposited on the tongue and fatty perception during oral processing of emulsion-filled gels.

Dynamics of Formation and Sensory Perception of Protein Oral Coatings

In preparation

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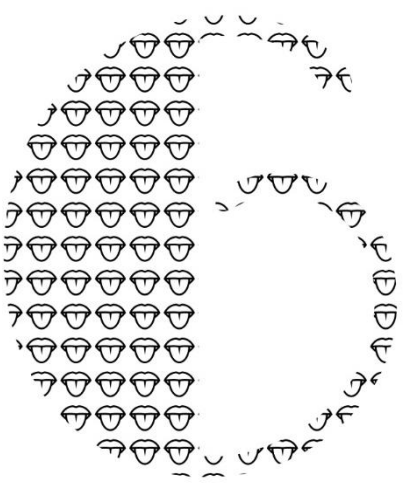

Abstract

The aim of this study was to determine the influence of protein content, in-mouth protein behavior and presence of thickener on the formation dynamics and sensory perception of protein oral coatings. Protein coatings were collected from the front and middle part of the anterior tongue (n=15 subjects) using cotton swabs after subjects orally processed model protein solutions for different time periods. Protein concentration of the coating (mass protein/mass coating) was quantified with the Lowry method. Sensory perception was evaluated at different oral processing time points for sweetness, creaminess, astringency and thickness. Protein concentration of the coatings deposited on the tongue surface reached its maximum after 3s of orally processing the protein solutions. Increasing the oral processing time further did not change the amount of protein deposited on the tongue. With increasing protein content of the stimuli, the amount of protein deposited on the tongue increased. Proteins differing in in-mouth behavior (proteins flocculating with saliva *vs.* proteins not flocculating with saliva) revealed similar dynamics of the formation of oral protein deposits. Addition of a thickener to the protein solution decreased oral protein deposition. Protein deposition influenced sensory perception mainly due to protein-saliva interactions and lubrication properties. We conclude that protein oral coatings behave similarly to oil oral coatings with respect to the kinetics of the formation of the oral coating.

Keywords: protein oral coatings, lubrication, perception, Na-caseinate, lysozyme, xanthan gum

1. Introduction

Oral coatings can be defined as the residues of food and beverages remaining in the oral cavity after the consumption of foods (de Wijk *et al.*, 2006). Oral coatings are known to influence after-feel perception of food, which is an important factor influencing food choice and acceptance.

Several methodologies have been described to quantify oral coatings (De Jongh and Janssen, 2007; Pivk *et al.*, 2008; de Wijk *et al.*, 2006) and often the amount of oral coating has been related to sensory perception (Prinz *et al.*, 2006; Kupirovič *et al.*, 2012; Camacho *et al.*, 2014). Lipid content of oral coating has been quantified by rinsing the oral cavity and subsequently determining the turbidity of the spat out solution as a measure of lipid content (de Wijk *et al.*, 2006). de Jongh and Janssen (2007) used attenuated total reflection IR (ATR-IR) spectroscopic analysis to analyse swabs containing oral coatings taken from distinct parts of the oral cavity over time. With this approach, the ratio of fat, protein and carbohydrate contents of oral coatings of different foods were determined. In contrast to the turbidity method, ATR-IR provides information about the relative chemical composition of the oral coatings. Nevertheless, without calibration it is not possible to quantify the amount of each ingredient deposited on the tongue. Pivk *et al.*, (2008a) used filter papers to extract oral coatings from the tongue formed by oils containing curcumin as a hydrophobic, fluorescent dye. The fluorescence intensity of the extracts was measured to quantify lipid content. It was not possible to fully recover all lipids from the tongue surface using this method. Therefore, Pivk (2008a), developed an *in vivo* method to characterize the thickness of oral coating by fluorescence measurements. The *in vivo* fluorescence method provided a direct measure of oral oil coatings thickness without damaging any of its components. Camacho *et al.*, (2014) developed the i*n vivo* fluorescence methodology further by determining calibration lines relating fluorescence intensity to oil fraction deposited on the tongue using pig´s tongues at 37.5°C to mimic oral conditions, and by using o/w emulsions as a more realistic food model

than pure oil (as used in previous research). Most of the methodologies described in literature predominantly focus on quantifying oil deposition in the oral cavity. As the formation of oral coatings is directly related to the consumption of foods which contain other macronutrients than oil, for example proteins, the deposition of proteins on the tongue surface can lead to the formation of a protein oral coating and consequently influence sensory perception.

In-mouth behavior of proteins was reported to affect after-feel and after-taste perception of model foods (Vingerhoeds *et al.*, 2005, Camacho *et al.*, 2014). After-feel creaminess and fattiness of lysozyme stabilized o/w emulsions under the clearance period (*i.e.* period when the oral coating was being washed away from the tongue) was less intense compared to Na-caseinate stabilized o/w emulsions, although the amount of oil deposited on the tongue was higher for the lysozyme stabilized o/w emulsions. This effect was suggested to be due to the difference in in-mouth behavior caused by the different proteins. Lysozyme forms complexes with salivary proteins by electrostatic interactions between the positively charged lysozyme and the negatively charged salivary biopolymers (Silletti *et al.*, 2007). The saliva induced flocculation of the o/w emulsion droplets reduces the lubrication of saliva and increases friction in mouth which leads to astringent and rough after-feel (Vingerhoeds *et al.*, 2005). On the other hand, Na-caseinate as a negatively charged protein does not interact with salivary biopolymers, creating no saliva induced flocculation.

Texture perception of foods is affected by lubrication through the different oral processing stages. Rubbing and squeezing the food between tongue and palate are important for the detection of sensations such creaminess or slipperiness where the food can act as a lubricant and reduce the friction, thus increasing lubrication, between the two interacting surfaces (Prakash *et al.*, 2013). Lubrication in mouth can be influenced by the addition of thickeners in foods. Van Aken *et al.*, (2011) found that the o/w emulsions after-feel perception of attributes such as coating and creamy increased when arabic gum was added to o/w emulsions. Camacho *et al.*, (2015) found that although the addition of xanthan gum to o/w emulsions

decreased the oil fraction deposited on the tongue, after-feel fatty film perception was higher either with addition of xanthan gum (corresponding to low oil deposited on tongue) or no addition of xanthan gum (corresponding to high oil deposited on tongue). This effect was attributed to the lubricating layer created by the xanthan gum.

In summary, the influence of oil and protein content, in-mouth behavior of protein stabilized o/w emulsions and thickening agents on the formation and clearance of oil oral coatings has been studied extensively. In contrast, little is known about the dynamics of formation and clearance of protein oral coatings. The aim of this study was to determine the influence of protein content, in-mouth protein behavior and presence of thickeners on the dynamics formation of protein oral coatings and sensory perception of protein solutions. To identify the individual importance of the protein on the oral coatings, this study used olutions of proteins, to minimize the interference with other macronutrients. Protein content (3% (w/w) Na-caseinate *vs.* 6% (w/w) Na-caseinate), in-mouth behavior of protein (3% (w/w) Lysozyme *vs.* 3% (w/w) Na-caseinate) and addition of thickener (3% (w/w) Na-caseinate with 0.2 % (w/w) xanthan gum *vs.* 3% (w/w) Na-caseinate with no xanthan gum) were varied. Cotton swabs were used to collect the oral coatings from the front and middle part of the anterior tongue. Protein content of the collected coating was subsequently determined using the Lowry method. A panel of n=15 untrained subjects processed orally the protein solutions for different times (t=3, 9, 15 and 30s) and sweetness, creaminess, astringency and thickness intensity of the protein coatings were assessed.

2. Materials and Methods

2.1 Materials

Na-caseinate (Excellion sodium caseinate S, DMV International, The Netherlands, protein content: 91%), lysozyme hydrochloride (The Protein Company, Belgium, protein content: > 99%), xanthan gum (Keltrol Advance

Performance, CP Kelco, Denmark), NaOH (Merck, Germany) and bottled mineral water (C1000, purchased from local retailer) were used. All ingredients were food grade.

2.2 Preparation of Protein Solutions

Four protein solutions were prepared: 3% (w/w) Na-caseinate, 6% (w/w) Na-caseinate, 3% (w/w) Na-caseinate with 0.2% (w/w) xanthan gum and 3% (w/w) lysozyme. The concentration of 0.2% (w/w) xanthan gum was chosen to considerably increase the viscosity compared to the % (w/w) Na-caseinate. A 6% (w/w) Na-caseinate stock solution was prepared at room temperature by dissolving the protein in water using a magnetic stirrer (IKA RW20 Digital Stirrer). A 0.4% (w/w) xanthan gum solution was prepared by dissolving xanthan gum in water at 70°C while stirring.

The 6% (w/w) Na-caseinate stock solution was diluted (1:1) with water to prepare the 3% (w/w) Na-caseinate solution. The 6% (w/w) Na-caseinate stock solution was diluted (1:1) with 0.4% (w/w) xanthan gum solution to prepare the 3% (w/w) Na-caseinate with 0.2% (w/w) xanthan gum solution. The 3% (w/w) lysozyme sample was prepared by dissolving lysozyme hydrochloride in water using a stirring plate at room temperature. The lysozyme solution was brought to a pH of 6.8 by adding 1M NaOH.

2.3 Rheological Characterization of Protein Solutions

Flow curves were determined for all solutions in duplicate at 20°C at shear rates ranging from 0.01 to 1000s^{-1} using a rheometer (Anton Paar GmbH, Physica MCR 301) equipped with double gap geometry.

2.4 Sensory Study

Selection of Attributes

A Check All That Apply (CATA) questionnaire was used by 20 untrained subjects (14 women and 6 men, mean age of 23.4±2) to select the attributes used for the

sensory profiling of the protein solutions. The CATA questionnaire included 12 attributes describing texture, flavor and taste (**table 6.1**). The order of the attributes on the questionnaire and presentation order of the four protein solutions were randomized between subjects. Subjects were instructed to sip 20 mL of each solution, swirl it in their mouth and expectorate it. After expectorating the protein solution, subjects were instructed to check all attributes that describe sensory perception of the stimulus. Each protein solution was presented to the subjects (n=20) in duplicate.

Table 6.1. List of sensory attributes used in CATA questionnaire for four protein solutions.

Attribute	Definition
Creamy	Range of sensations typically associated with fat content, such as full, compact, smooth, not rough, not dry, with a velvety (not oily) coating.
Rough	Degree which the product remaining in mouth creates a rough sensation on the tongue typically caused by nuts, spinach and wine.
Dry	Degree to which product remaining in mouth appears to absorb saliva on the tongue
Astringent	Degree to which the product remaining in mouth generates an astringent sensation. *Astringent:* complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins
Thick	Thickness of the coating remaining on the tongue after it is compressed via up-and-down motions against palate.
Slippery	Ease to slide the tongue over the palate
Milky	Degree to which the product remaining in mouth creates a flavour associated to fresh milk
Boiled Milk	Degree to which the product remaining in mouth creates a flavour associated to boil milk

Attribute	Definition
Sweet	Degree to which the product remaining in mouth creates a taste associated to sugars
Sour	Degree to which to which the product remaining in mouth creates a taste associated to acids (e.g. lemon, vinegar)
Bitter	Degree to which to which the product remaining in mouth creates a taste associated to compounds such caffeine (coffee, tea), theobromine (dark chocolate)
Salty	Degree to which product remaining in mouth creates a taste associated to salts (e.g. NaCl, table salt)

Selection of Subjects

To select the subjects for the sensory profiling of the protein solutions after orally processing for different time periods, a screening session was performed with 20 subjects (12 women and 8 men, mean age of 23.7±2.6). This session involved an introduction to the definition of the sensory attributes which were selected based on the outcomes of the CATA (creaminess, thickness, astringency and sweetness) and the sensory scale (100 mm Visual Analogue Scale (VAS) anchored at the ends with "not at all" and "very much"). Subjects were familiarized with the four protein solutions by tasting them, and a group discussion addressing differences between samples and attributes was performed.

The screening session ended with individual tests. In the first test, subjects were asked to process in mouth each of the four protein samples (20 mL) for 30 s, expectorate and rate the intensity of the sensory attributes (creaminess, thickness, astringency and sweetness) on the VAS scale for each sample. In the second test, subjects were asked to process in mouth each of the four protein samples (20 mL) for 3 s, expectorate and rate the sensory attributes for each sample (in duplicate). The duration of the oral processing times was chosen in order to familiarize the subjects with the shortest and the longest oral processing time used in the subsequent sensory sessions.

Fifteen untrained subjects (10 females and 5 males, mean age of 23.6±1.9) were selected to continue the study based on their understanding of the attributes, use of the scale and availability. All subjects signed a consent form and received financial compensation for their efforts.

Sensory Profiling of Protein Solutions

Fifteen untrained subjects performed sensory profiling of the four protein solutions. Subjects sat in individual sensory booths at room temperature with white light. Subjects were asked to orally process 20 mL of each protein solution for different times (t=3, 9, 15 and 30 s) at room temperature. The solution was expectorated and immediately afterwards the intensity of the four selected attributes (**table 6.2**) was rated using a 100 mm VAS scale. In total 16 conditions (4 protein solutions x 4 oral processing times) were tested in triplicate by the subjects (n=15) resulting in 48 measurements per subject. Sessions lasted for 1h and subjects participated in 3 sessions over a period of 2 weeks. During each session, 16 conditions were assessed by each subject with a break of 5 min after 8 conditions. Between the tasting of solutions, subjects cleaned their palates with water and crackers. A completely randomized design was used randomizing the 48 conditions over subjects and sessions.

Table 6.2. Definition of sensory attributes and evaluation protocol

Attribute	Definition	Evaluation protocol
Creaminess	Range of sensations typically associated with smooth, soft velvety (not oily) coating.	After expectorating the sample, slide the tongue on the palate.
Thickness	Thickness of the coating left on the tongue	After expectorating the sample, compress the tongue via up and down motions against palate.

Attribute	Definition	Evaluation protocol
Astringency	Puckering and dry sensation left on the tongue after swallowing the food. Typically caused by products like wine, tea and spinach.	After expectorating the sample, evaluate the astringency perceived in the mouth
Sweetness	Degree to which product leaves a taste associated to sugars	After expectorating the sample, evaluate the sweet taste perceived in the mouth

2.5 Collection and Quantification of Protein Oral Coatings

Cotton swabs (Model 520CS01, COPAN Flock Technologies, Italy) were used to collect oral coatings and saliva. For each oral protein coating's collection, six sterile cotton swabs were pre-weighed. Three swabs were used to collect saliva from the subjects tongue prior to the ingestion of any stimulus to quantify the amount of protein present in the saliva of each of the subjects (base line measurement). The remaining three swabs were used to collect the oral protein coating after orally processing the protein solutions for different times to quantify the amount of protein present in the oral protein coating. Mass of collected coating was determined by weighing the cotton swabs before and after the collection of the coating from the tongue.

The same fifteen untrained subjects who participated in the sensory evaluations were used to collect the protein oral coatings. The same 48 conditions as in the sensory study were tested (4 protein solutions x 4 oral processing times x 3 replicates). A completely randomized design was used randomizing the 48 conditions over subjects and sessions. During each session of 1 h, 8 conditions were tested. Each subject participated in 6 session of coating collection over 5 weeks.

Before orally processing the protein solution, subjects were asked to rinse their mouth with water. Saliva on their tongue was collected, with cotton swabs from the anterior middle until the anterior front part of the tongue by gently rubbing the cotton swabs on the tongue surface. The swabs with the saliva were weighted to quantify the mass of saliva collected. The swabs were then transferred to a cuvette with 1mL of cold PBS 10X solution (Sigma, USA) (3 swabs/cuvette). The swabs were kept in the cuvette for about 5 min and later discarded. Then, subjects were asked to ingest 20 mL of each protein solution and orally process it for different times. Subjects expectorated the protein solution and the oral protein coating was collected using the same procedure as in the saliva collection. After each sample, the subjects cleaned their mouth with water and crackers.
After the collection of saliva and oral protein coating, the cuvettes were kept at 4°C for a maximum of 4h and then the protein content was quantified using the Lowry method.

To quantify the amount of protein, the cuvettes with either saliva or protein oral coating in the PBS solution, were centrifuged (Eppendorf 5430R Centrifuge) for 5 min at 20°C at 14000 rpm to dispose cellular debris. The supernatant was analyzed using a modified Lowry Protein Assay Kit (Thermo Scientific, USA) following the instructions of the manufacturer. The absorbance was measured at 750 nm using a UV spectrophotometer (Cary 50 Bio UV.Vis spectrophotometer). To determine the concentration of protein, two calibration lines were made with either Na-caseinate or lysozyme solutions. To correct for the presence of salivary proteins, the quantified protein in the saliva was subtracted from the protein on the coating. Protein concentration of the protein oral coating is expressed from here onwards as mass protein/mass oral coating.

2.6 Statistical Data Analysis

SPSS® Statistics version 21 was used for the statistical data analysis. Protein coating concentration data was normalized with a square root transformation. Outliers (z>2) were removed from the data. The effects of sample (within subject factor: 3% (w/w) Na-caseinate, 6% (w/w) Na-caseinate, 3% (w/w) Na-caseinate with 0.2% xanthan gum or 3% (w/w) lysozyme), oral processing time (within subject factor: 3, 9, 15 and 30s) and the interaction on the formation of the coating were tested with a repeated-measures ANOVA. The assumption of sphericity was verified by Mauchly's test being violated for oral processing time and the interaction effect. Therefore, the Greenhouse-Geisser correction was applied.
Sweetness and astringency data were normalized with a log (x+1) transformation The effects of sample (within subject factor: 3% (w/w) Na-caseinate, 6% (w/w) Na-caseinate, 3% (w/w) Na-caseinate with 0.2% (w/w) xanthan gum or 3% (w/w) lysozyme), oral processing time (within subject factor: 3, 9, 15 and 30 s) and the interaction between creaminess, thickness, astringency and sweetness were tested with a repeated-measures ANOVA. When the assumption of sphericity was violated the Greenhouse-Geisser correction was applied. When significant main effects were found Bonferroni tests were performed. A significance level of $p<0.05$ was chosen.

3. Results

3.1 Rheological Characterization of Protein Solutions

Table 6.3 shows the viscosity of the four protein solutions. All solutions displayed shear thinning behavior with differing levels of shear thinning. The viscosity of the solutions at a shear rate of $63s^{-1}$ were extracted from the flow curves. The shear rate of $63s^{-1}$ was chosen as it is in the range of shear rate that have been suggested to occur in mouth (50-100 s^{-1}).

Table 6.3. Viscosity at a shear rate of $63s^{-1}$ of protein solutions Average of replicates ± standard deviation are shown.

Solution	Viscosity at shear rate $63s^{-1}$ (mPa.s)
3% Na-Cas	2.2 ± 0.01
6% Na-Cas	5.3 ± 0.01
3% Na-Cas with 0.2% Xanthan	37.1 ± 1.60
3% Lys	1.4 ± 0.04

3.2 Sensory Perception of Protein Coatings

Selection of Sensory Attributes using CATA

Three criteria were considered to select the sensory attributes used in the profiling study: a) attributes which describe the sensory properties of the four protein solutions and allow the differentiation between different protein solutions, b) attributes which have high frequency of selection by the untrained subjects in the CATA questionnaire, and c) attributes which are easily understandable to untrained subjects. **Figure 6.1** depicts the results of the CATA questionnaire.

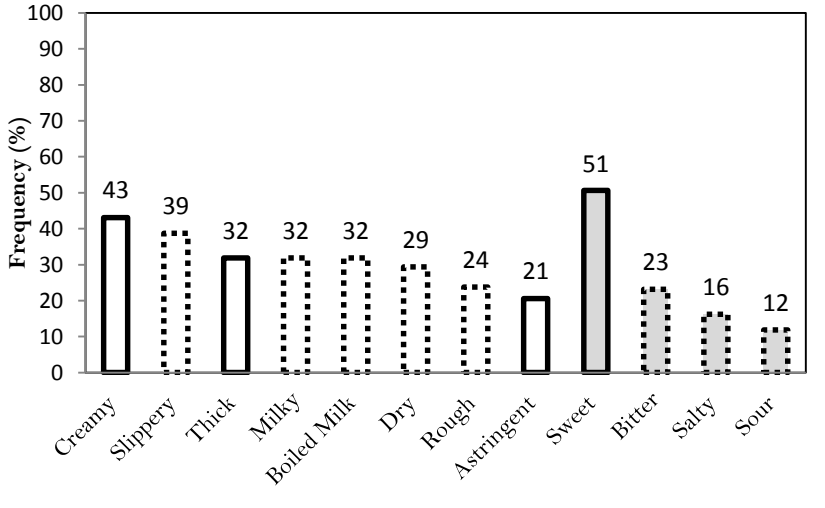

Figure 6.1. Frequency of selection of sensory attribute by CATA questionnaire. Bars represent the percentage of times that an attribute was chosen for the four protein solutions. Bars with continuous

line represent attributes selected for the sensory study. Each protein solution was presented to the subjects (n=20) in duplicate.

The two attributes most frequently selected were creaminess and sweetness, thus these two attributes were selected for the sensory profiling. In order to discriminate samples with higher viscosity (6% (w/w) Na-Cas and 3% (w/w) Na-Cas with 0.2% (w/w) xanthan) from samples with lower viscosity, the attribute thickness was chosen. Thickness was the attribute with the highest selection ratio (high viscosity samples/low viscosity samples): 41 selections for the high viscosity samples versus 10 selections for the low viscosity samples. Astringency was selected in order to discriminate the 3% (w/w) Lys sample, which had the highest attribute selection ratio (3% (w/w) Lys/ (3% (w/w) Na-Cas + 6% (w/w) Na-Cas + 3% (w/w) Na-Cas with 0.2% (w/w) xanthan): 27 selections for 3% (w/w) Lys versus 6 selections for the three remaining samples.

Dynamic Sensory Profiling of Protein Oral Coatings

Figure 6.2 shows the sensory perception of the protein oral coatings as function of oral processing time. For the four attributes selected, sample had a significant main effect on the perception of the four sensory attributes (creaminess: $[F(2.3, 102)=159.8, p<0.001]$, thickness: $[F(1.5, 66.7)=12.3, p<0.001]$, astringency: $[F(2.4, 105)=350.4, p<0.001]$ and sweetness $[F(1.6, 70.1)=112.8, p<0.001]$). No significant main effect of oral processing time nor interaction on the oral coating's perception was found for any attribute.

With increasing viscosity of the protein solution (**table 6.3**), the perception of thickness and creaminess increased. The coating formed by the protein solution with highest viscosity (3% (w/w) Na-Cas with xanthan) was scored as the most creamy ($p<0.001$) and the most thick ($p<0.05$) at every oral processing time. Although non-significantly, the coatings formed by the 6% (w/w) Na-Cas tended to be perceived as creamier and thicker ($p=0.051$) than the coatings formed by 3% (w/w) Na-Cas. The coating formed by 3% (w/w) Lys solution was perceived as significantly less creamy ($p<0.001$) compared to the remaining samples, and less thick to the coatings formed by 6% (w/w) Na-Cas and 3% (w/w) Na-Cas with

xanthan ($p<0.05$) at every oral processing time. With increasing oral processing time, thickness and creaminess did not change significantly for any of the four protein coatings.

The coatings formed by the 3% (w/w) Na-Cas and 6% (w/w) Na-Cas were scored similarly in astringency. The coating formed by the 3% (w/w) Na-Cas with xanthan was perceived significantly less astringent than all other protein coatings ($p<0.001$). The coatings formed by the 3% (w/w) Lys sample were scored as 3 folds higher astringent intensity compared to the remaining samples ($p<0.001$).

The coatings formed by the 3% (w/w) Lys sample were perceived as 4x higher in sweetness intensity compared to the remaining samples ($p<0.001$). The three other protein samples were not perceived as significantly different.

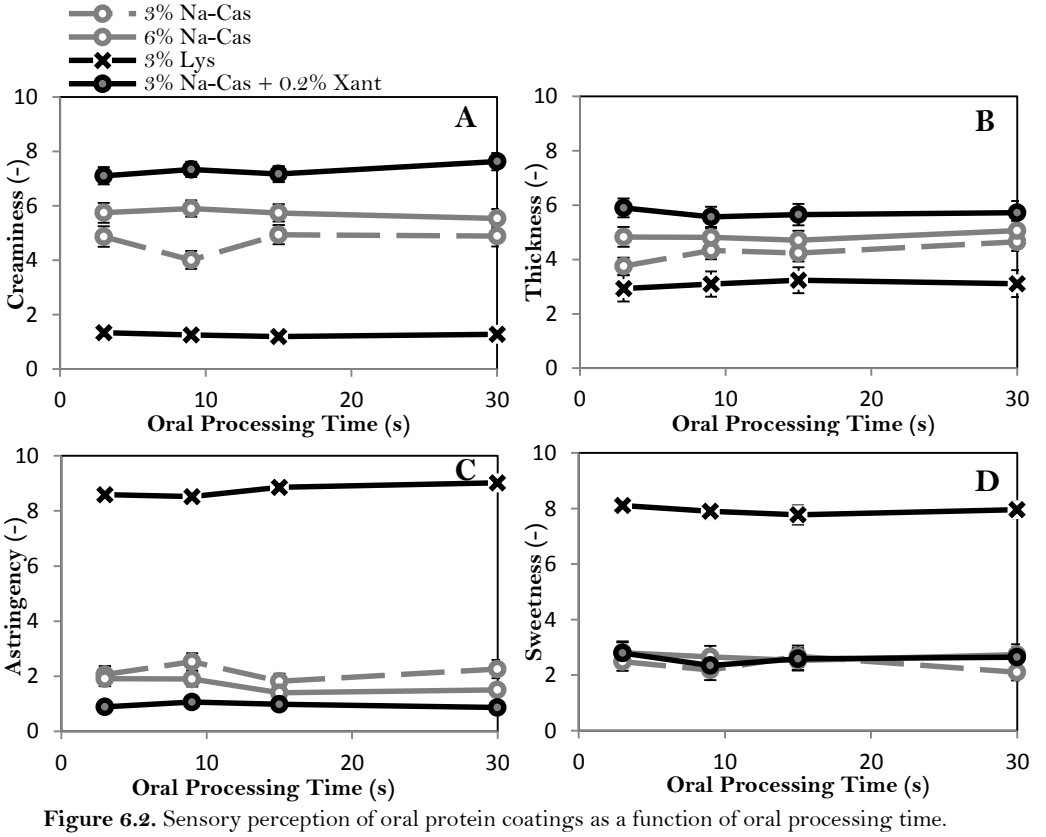

Figure 6.2. Sensory perception of oral protein coatings as a function of oral processing time.

A- Creaminess. B- Thickness. C- Astringency. D- Sweetness. Each data point represents the average of n=15 subjects and 3 replicates. Error bars represent standard error. Lines are drawn to guide the eye.

3.3 Dynamics of Formation of Oral Protein Coatings

Figure 6.3 depicts the protein deposition on the front and middle part of the anterior tongue immediately after expectoration of the protein solutions as a function of oral processing time. Sample ($F_{[2.5,\ 111]}=93.9$, $p<0.001$), time ($F_{[3,132]}=6.41$, $p<0.001$) and the interaction ($F_{[9,\ 396]}= 2.98$, $p=0.002$) have a significant main effect on the protein concentration deposited on the tongue surface.

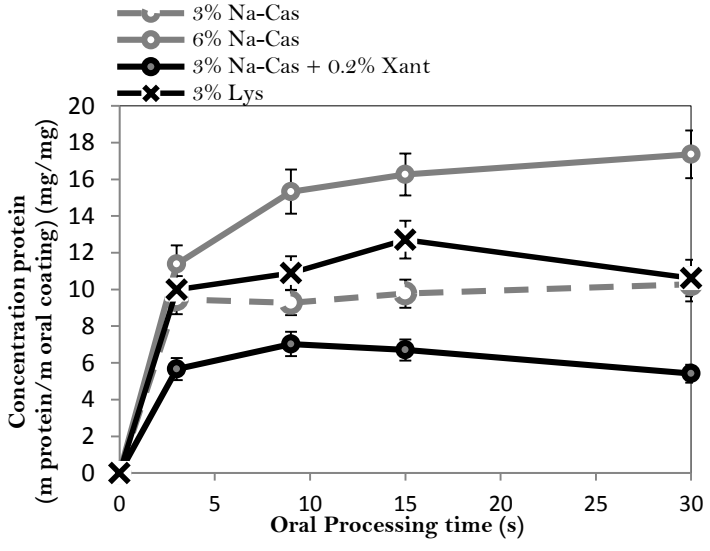

Figure 6.3. Concentration of protein (mass of protein/mass oral coating) deposited on the front and middle part of the anterior tongue as a function of oral processing time. Each point represents the average of n=15 subjects and 3 replicates. Error bars represent the standard error. Lines are drawn to guide the eye.

The oral processing time affected the protein deposition created by the 6% (w/w) Na-Cas solution. The longer the 6% (w/w) Na-Cas solution was processed in mouth, the higher the oral protein deposition. Oral processing of this solution for 3 s creates a significantly lower protein deposition ($p< 0.001$) compared with the other oral processing times. For the other three protein solutions (3% (w/w) Na-

Cas, 3% (w/w) Na-Cas with 0.2% (w/w) xanthan and 3% (w/w) Lys), the oral processing time did not affect the protein deposition.

After 3s of oral processing, the 3% (w/w) Na-Cas with 0.2% (w/w) xanthan solution created a significantly lower protein deposition on the coating ($p<0.001$) compared with all other protein solution. The 3% (w/w) Na-Cas, 3% (w/w) Lys and 6%(w/w) Na-Cas solutions did not differ significantly in protein deposition (t=3s).

The 3% (w/w) Na-Cas with 0.2% (w/w) xanthan solution revealed a significantly lower protein deposition in the coating across all oral processing times ($p<0.001$) compared with the remaining solutions. Protein deposition of the 6% (w/w) Na-Cas solution was significantly higher than for the other thre protein solutions from 9 s onwards ($p<0.001$). Protein deposition of 3% (w/w) Na-Cas and 3% (w/w) Lys solutions was not significantly different independent of oral processing time.

4. Discussion

The aim of this study was to determine the influence of protein content, in-mouth protein behavior and presence of thickeners on the formation dynamics of protein oral coatings and sensory perception of protein solutions.

Prolonging oral processing time from 3 s to 30 s increased significantly the protein deposition on the tongue created by the protein solution with the highest protein content (6% (w/w) Na-Cas). The deposition of the protein in the oral coating on the tongue was fast with the maximum protein deposition being reached after an oral processing time of 3 s. A oral processing time of 3 s corresponds to the natural drinking time of a sip of 20 mL of a dairy-based beverage. Prolonging oral processing times up to 30 s did not lead to a further increase in protein deposition on the tongue for the four protein solutions. This is in accordance with other studies (Chapter 4) demonstrating that oral processing of oil/water (o/w) emulsions for longer oral processing times than 3 s does not lead to an increase of oil deposited on the tongue. On the other hand, de Jongh *et*

al., (2007), found that swirling dressings for longer times in the mouth (5s *vs.* 20s) created higher deposition of protein/carbohydrates on the middle of the tongue. This difference is likely due to the readily availability of the macronutrients in the liquid solutions used in our study and in the study of Camacho *et al.* (2014), which are more easily in contact with the tongue papillae facilitating their fast deposition on the tongue in contrast to the soft semi-solids used by de Jongh *et al.*, (2007).

The 6% (w/w) Na-Cas solution showed the highest protein deposition compared to the other protein solution which contained 3% (w/w) protein. This result is in accordance with studies on oil coatings, which reported that with increasing oil content, the oil fraction deposited on the tongue increases (Camacho *et al.*, 2014, 2015, Pivk *et al.*, 2008b). This suggests that the deposition of oil and protein follows a similar behavior despite their difference in chemical properties.

The 3% (w/w) Na-Cas and 3% (w/w) Lys solutions were found to form oral protein deposition content that are not significantly different from each other. Lysozyme can form flocs with saliva due to electrostatic interaction between the positive charged lysozyme and the negative charged salivary proteins (Silletti *et al.*, 2007). On the other hand, Na-caseinate creates no visible interaction with saliva, *i.e.* no flocculation (Camacho *et al.*, 2015). Although the two proteins have different behavior in mouth, this did not affect the amount of protein deposition on the tongue. This is in agreement with results by Camacho *et al.*, (2015) found that the oil fraction on the tongue after the consumption of a Na-caseinate stabilized o/w emulsion compared to a lysozyme stabilized emulsion was similar. The findings suggest that both ingredients with electrostatic interactions and electrostatic repulsions have similar deposition kinetics on the tongue. Other studies showed the importance of different forces on the adhesion of different samples on tongue-like conditions. Malone *et al.*, (2003) studied the interactions between emulsions stabilized either with a non-ionic emulsifier (Tween 60) or a positive emulsifier (chitosan) on a mucin film (with negative charge) using evanescent wave spectroscopy. Results showed that oil droplets stabilized with

chitosan were adsorbed onto the mucin film, while oil droplets stabilized with Tween 60 did not show any adsorption. This was suggested to be related to electrostatic interactions between the oil droplets and the mucin film. Dresselhuis *et al* (2008) identified the importance of the spreading of the o/w/ emulsions on the surface for sensory perception in addition to the adhesion of o/w emulsions onto the surface,. Dresselhuis *et al.*, (2008) studied the effect of electrostatic, steric and hydrophobic forces between protein stabilized o/w emulsions and hydrophobic and hydrophilic glass surfaces using a flow cell with light microscopy. The adhesion and spreading of the emulsion droplets was enhanced when the electrostatic and steric forces were reduced and hydrophobic attraction between droplets and the glass surface occurred. It seems that the adhesion onto a solid surface dependents on physical and chemical forces between the surface and the adhering material. Nevertheless, according to our results the underlying mechanisms for the adherence of an ingredient to the tongue surface are based mainly on mechanical interactions. It is possible that under in-mouth conditions, the shear of the tongue against the palate has a stronger influence on adhesion and spreading compared to chemical-physical forces between the tongue surface and the food adhering to it. Nevertheless, these forces seem to have an impact on the clearance of the coating. Camacho *et al.*, (2015) demonstrated that oil droplets deposited on the tongue were more rapidly washed away when the droplets were stabilized by Na-Cas (no interaction with the mucus layer on the tongue) compared to when the droplets were stabilized by Lys (electrostatic interactions with the mucus layer on the tongue).

The presence of 0.2% (w/w) xanthan gum in the 3% (w/w) Na-Cas solution created a significantly lower protein deposition compared with the 3% (w/w) Na-Cas sample without xanthan. This is in agreement with previous studies which have shown that the presence of a thickener in o/w emulsions decreased the oil deposition on the tongue compared to o/w emulsions without thickeners. This is likely due to the more viscous matrix created by the thickener which entraps the macronutrients and limits their deposition between the papillae.

The perception of creaminess, thickness, sweetness and astringency of the protein coatings was not influenced by oral processing time. The deposition of protein coatings reached it maximum already after short oral processing times (3 s). No considerable change in the amount of protein deposited on the tongue surface is observed with increasing oral processing time, thus perception intensity is also not affected.

Creaminess and thickness intensity of the four protein solutions increase with increasing viscosity. Several authors have related the creaminess and thickness to viscosity (Kilcast and Clegg, 2002; Akhtar *et al.*, 2005; Vingerhoeds *et al.*, 2009). Additionally, in line with this study, Camacho *et al.*, (2015) and Vingerhoeds *et al.*, (2009) also found an increase in creaminess and thickness with the addition of a thickener to o/w emulsions. It was suggested that the thickener forms a lubricating layer decreasing the friction between oral surfaces Malone *et al.*, (2003) observed a decrease in the friction coefficient with an increase of guar gum content in aqueous solutions. It was suggested that high viscosity can lower friction due to the formation of a lubricating layer between the contacting surfaces. Although the 3% (w/w) Na-Cas solution with 0.2% (w/w) xanthan had the lowest oral protein deposition, the lubrication properties of xanthan significantly contributed to the perception of creaminess and thickness.

The perception of the coatings formed by the 3% (w/w) Lys solution were perceived as more astringent and sweet in comparison to the Na-caseinate solutions, which were perceived more creamy and thick. The sweeter and more astringent perception of lysozyme was expected. Lysozyme has a sweet taste (Masuda *et al.*, 2005), which explains the high intensity of sweetness in comparison to Na-caseinate solutions. Furthermore, Vingerhoeds *et al.*, (2009) found that lysozyme stabilized emulsions were related to astringency perception. It was suggested that the astringency perception could be due to the interaction between lysozyme and saliva, which creates big agglomerates and increases the friction in-mouth, in a similar way to tannins. Further, the interactions with saliva reduce the content of saliva protein which would in turn reduce the lubrication

behaviour. Moreover, studies found that positively charge proteins are perceived more astringent in comparison to negatively charge proteins (Beecher *et al.*, 2008; Vardhanabhuti *et al.*, 2010; Ye *et al.*, 2011).

Dresselhuis *et al.*, (2007) suggested that the similar friction coefficient found between two different concentrations of o/w emulsions was due to only a small quantity of oil being needed to form a lubricant layer. The same principle might be applied for proteins, in which the mucosa surface could already be well lubricated with the 3% (w/w) Na-Cas, and thus not provoking a decrease on friction when more protein (6% (w/w) Na-Cas) is present.

In conclusion, this study shows that protein oral coatings influence sensory perception and in-mouth lubrication. Protein oral coatings behave similarly to oil oral coatings with respect to the kinetics of the formation of the oral coating (i.e. the adherence dynamics to the tongue surface). Protein coatings reach maximum deposition on the tongue surface after 3 s of oral processing and do not change with prolonged oral processing time. Sensory perception of the deposition formed by the protein solutions follows a similar trend in time. Proteins with different behavior in-mouth do not show different protein deposition on the tongue. This suggests that protein oral coatings are formed mainly due to mechanical effects *(i.e.*, shear between tongue and palate) and less due to electrostatic interactions or colloidal forces between the tongue surface and the protein solution. Na-caseinate content and viscosity of the solution influenced the protein deposition on the tongue with higher protein availability (higher content, and no thickener matrix) creating higher protein deposition on the tongue. Protein deposition on the tongue influenced sensory perception, possibly due to protein-saliva interactions and lubrication in mouth.

Abbreviations Used:

Na-Cas: Na-caseinate (Sodium caseinate), Lys: Lysozyme, xanthan: xanthan gum, o/w: oil in water

Just Noticeable Differences and Weber fraction of Oral Thickness Perception of Model Beverages

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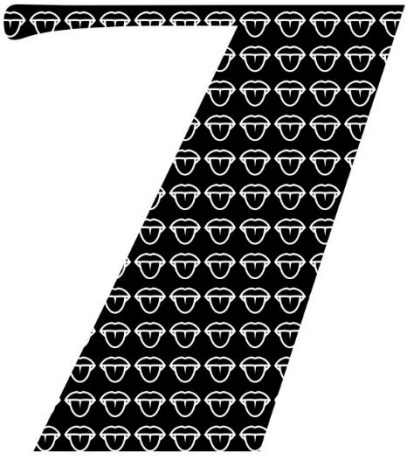

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Abstract

Rheological properties of beverages contribute considerably to texture perception. When developing new beverages, it is important to have knowledge on the smallest differences of viscosity which a consumer can discriminate. Thickness is the sensory attribute most commonly used to describe the viscosity of beverages. The aim of this study was to determine the Just Noticeable Differences (JND's) of oral thickness perception and the Weber fraction (K) of Newtonian model stimuli (maltodextrin solutions). JND's were determined using the method of constant stimuli with five reference stimuli ranging in viscosity from 10 to 100 mPa.s. JND's increased with increasing viscosity of the reference stimulus. The Weber fraction (K) for oral thickness perception of model beverages was K=0.26 for the studied viscosity range. The Weber fraction for oral thickness perception is comparable to Weber fractions reported in literature for perception of kinesthetic food firmness and spreadability, creaminess, sourness and bitterness perception. This demonstrates that the human sensitivity towards oral discrimination of thickness of liquid stimuli is comparable to the human sensitivity towards discrimination of specific texture properties and specific taste stimuli.

Keywords: Just Noticeable Differences, Weber fraction, thickness, viscosity, maltodextrin

1. Introduction

Texture contributes considerably to the sensory perception and appreciation of beverages. When assessing texture of beverages the rheological properties of the beverage play an important role. Thickness is usually used as the sensory attribute describing and being directly related to the viscosity of beverages (Stanley and Taylor 1993). Thickness can be assessed in mouth by judging the resistance of the sample to flow, the rate of flow and the amount of force needed to translocate the fluid in the mouth (Akhtar *et al.*, 2005). When two beverages or liquid stimuli are perceived as equally thick, it does not necessarily mean that the stimuli have the same viscosity. It can be the case that the difference in viscosity between the two stimuli is too small to be perceived differently in thickness. Sensory difference thresholds are usually studied by determining the Just Noticeable Difference (JND) of stimuli using the method of constant stimuli (Lawless and Heymann 2010). From JND's determined for various reference stimuli, the Weber fraction (K) can be obtained. The Weber fraction (K) is an index of the sensitivity of the sensory system to detect changes of a certain stimulus (Lawless and Heymann 2010). JND's have been determined for various sensory modalities including auditory stimuli (Aronson 1994), visual acuity (Huang *et al.*, 2008), tactile stimuli (Salada 2004) and car design (Hoffman, 1968, Mansfield 2000). In food sciences, the focus has been on the determination of taste and olfactory JND's (Schutz and Pilgrim 1957; Stone and Bosley 1965; McBride 1983; Goldstein 2010; Orellana-Escobedo *et al.*, 2012; Hoppert *et al.*, 2012). In contrast to the studies on JND's of taste and olfaction, little is known about JND's for texture perception of foods and beverages. A recent study reported JND's for creaminess perception when the apparent viscosity of dairy-based emulsions varied. Apparent viscosity variations were achieved by differing fat content or locust bean gum content in emulsions (Zahn *et al.*, 2013). Calculated Weber fractions were approximately K=0.20 (Zahn *et al.*, 2013). Rohm and Raaber (1992) studied the JND's and Weber fraction for kinesthetic firmness perception and

spreadability perception of edible fats by asking subjects to cut and spread different spreads with a knife (Rohm and Raaber 1992). As this study focused on the perception of kinesthetic food texture, it elegantly circumvented challenges related to the determination of JND's and Weber fractions for oral texture perception.

The method of constant stimuli consists of a series of directional 2-alternative forced choice tests (2-AFC) for which stimuli should differ only in a single sensory property. Consequently, when determining JND's of sweetness, sweetness should be the only perceptual difference between two stimuli of a pair while all other taste, flavor and texture properties should remain unchanged in order to avoid dumping effects. Changing a single textural property of foods and beverages without modifying other sensory properties such as taste and flavor remains a challenge and often requires the use of model foods. We hypothesize that this limitation is the reason why there are only few studies focusing on the determination of JND's of texture perception despite its important contribution to the appreciation of beverages and foods. This study contributes to filling this knowledge gap.

The aim of this study was to determine the JND's of oral thickness perception and the Weber fraction of Newtonian model stimuli using the method of constant stimuli. Model beverages varying in maltodextrin concentration with five constant, reference stimuli ranging in viscosity from 10 mPa.s to 100 mPa.s were assessed.

2. Materials and Methods

2.1 Materials Preparation and Characterization of Stimuli

An overview of the composition and viscosity of all stimuli is given in **table 7.1**. Aqueous solutions of maltodextrin were prepared by dissolving maltodextrin DE 6 (Glucidex IT6, Roquette, France) in demineralized water at room temperature. Vanilla aroma (Bourbon vanilla VLB concentrate, Pomona Aroma, The

Netherlands) was added to mask minor sweetness differences between stimuli varying in maltodextrin concentration and to increase palatability of stimuli. Concentration of vanilla aroma varied between 0.10% to 0.20% (w/w) depending on maltodextrin concentration. The concentration of vanilla aroma for each stimulus was chosen based on a sensory pre-test by three subjects to determine the vanilla aroma concentration so that stimuli varying in maltodextrin concentration were perceived as iso-intensive with respect to sweetness and vanilla flavor. Five reference stimuli differing in viscosity were used which are referred to in this paper as 10, 25, 50, 75 and 100 mPa.s. The precise viscosities of all stimuli are shown in **table 7.1**. The method of constant stimuli was used to determine the JND's. For every reference stimulus, six comparison stimuli were assessed of which three stimuli had a higher viscosity than the reference and three stimuli had a lower viscosity than the reference. Preliminary testing suggested greater sensitivity for thickness discrimination with increasing viscosity. Therefore, the relative viscosity differences between the reference and comparison stimuli (**table 7.1**) were chosen so that with increasing viscosity of the reference stimulus the relative difference in viscosity between reference and comparison stimulus decreased (experimental procedure similar to McBride 1983).

The viscosity of all stimuli was determined in duplicate using a Physica MCR 301 Rheometer (Anton Paar GmbH) at 20^0C at shear rates ranging from 1-1000 s^{-1} with a double gap geometry. The average viscosities were calculated for the shear rate range measured and are reported in **table 7.1**.

Table 7.1. Composition, viscosity and (aimed) viscosity difference relative to the reference of all stimuli.

Stimulus	Viscosity (mPa.s)	Viscosity difference relative to reference (%)	Aimed viscosity difference relative to reference (%)	Maltodextrin (% w/w)	Vanilla aroma (% w/w)
Reference 10 mPa.s					
Comparison 1	1.1±0.06	-89.2	-100	1.00	0.10
Comparison 2	3.2±0.13	-68.6	-66	11.0	0.10
Comparison 3	7.0±0.14	-31.4	-33	17.0	0.10
Reference	**10.2±0.14**	-	-	**20.0**	**0.10**
Comparison 4	13.8±0.05	35.3	33	22.0	0.10
Comparison 5	16.1±0.08	57.8	66	23.0	0.10
Comparison 6	20.5±0.15	101.0	100	25.0	0.10
Reference 25 mPa.s					
Comparison 1	6.0±0.06	-76.3	-75	16.0	0.10
Comparison 2	12.0±0.02	-52.6	-50	21.0	0.10
Comparison 3	18.1±0.11	-28.5	-25	24.0	0.10
Reference	**25.3±0.10**	-	-	**26.0**	**0.15**
Comparison 4	31.2±0.18	23.3	24	27.5	0.15
Comparison 5	38.8±0.13	53.4	50	29.0	0.15
Comparison 6	45.6±0.23	80.2	75	30.0	0.15
Reference 50 mPa.s					
Comparison 1	25.3±0.10	-49.4	-50	26.0	0.15
Comparison 2	33.2±0.12	-33.6	-33	28.0	0.15
Comparison 3	41.4±0.43	-17.2	-17	29.5	0.15
Reference	**50.0±0.47**	-	-	**31.0**	**0.15**
Comparison 4	60.6±0.33	21.2	17	32.0	0.15
Comparison 5	66.9±0.60	33.8	33	32.4	0.15
Comparison 6	75.0±0.78	50.0	50	33.3	0.15

Stimulus	Viscosity (mPa.s)	Viscosity difference relative to reference (%)	Aimed viscosity difference relative to reference (%)	Maltodextrin (% w/w)	Vanilla aroma (% w/w)
Reference 75 mPa.s					
Comparison 1	50.0±0.47	-33.3	-33	31.0	0.15
Comparison 2	60.6±0.33	-19.2	-22	32.0	0.15
Comparison 3	66.9±0.60	-10.8	-11	32.4	0.15
Reference	**75.0±0.78**	-	-	**33.3**	**0.20**
Comparison 4	84.3±0.94	12.4	11	34.0	0.20
Comparison 5	90.0±1.00	20.0	22	34.5	0.20
Comparison 6	98.8±0.83	31.7	33	35.0	0.20
Reference 100 mPa.s					
Comparison 1	75.0±0.78	-24.1	-25	33.3	0.20
Comparison 2	84.3±0.94	-14.7	-17	34.0	0.20
Comparison 3	90.0±1.00	-8.9	-8	34.5	0.20
Reference	**98.8±0.83**	-	-	**35.0**	**0.20**
Comparison 4	108.9±3.17	10.2	8	35.5	0.20
Comparison 5	114.2±1.08	15.6	17	36.0	0.20
Comparison 6	125.5±1.70	27.0	25	36.5	0.20

2.2 Method of Constant Stimuli

The method of constant stimuli is a difference test using a series of paired comparisons for measuring difference thresholds. In the method of constant stimuli, the test stimulus is always compared to a constant reference stimulus which is the middle point in a series of comparisons (Lawless and Heymann 2010). Fifteen untrained subjects (6 males, 9 females) with a mean age of 23 ±3 years were recruited for the study. Participants were asked not to eat or drink for at least one hour prior to the test. Participants gave written informed consent and received a financial compensation for their participation. Participants were seated in individual sensory booths at room temperature with white light. All stimuli

were prepared freshly on the day of the sensory session in a food grade environment. Five sessions of approximately 30 minutes were conducted. In each session one reference stimulus was assessed. The order of reference stimuli over the five sessions was randomized. All participants assessed the same reference stimulus in the same session. In each session one reference stimulus was compared with six comparison stimuli in duplicate. Therefore, each session consisted of 12 paired comparisons (2-AFC tests). All stimuli were coded with 3 digit numbers. Within one session, the presentation order of the 2-AFC's was randomized over participants. The first session started with a warm-up question to familiarize the subjects with the stimuli and with the questionnaire. The warm-up question consisted of the comparison between the stimuli of 10 and 75mPa.s which was excluded from the data analysis.

The 2-AFC tests were made using EyeQuestion (Version 3.9.7). Subjects were asked: "Which sample is thicker?". Thickness was defined as: "Thickness is the degree to which the fluid resists flow under an applied force in the mouth. Consider for example three fluids: water, drinking yoghurt and honey. Water has the lowest thickness, drinking yoghurt is thicker than water and honey is the thickest fluid of all three."

A volume of 20 mL of each stimulus was presented in 35 mL plastic cups at room temperature covered with a lid on a tray which was already present when the panelist entered the sensory booth. In order to standardize the effect of α-amylase on the enzymatic degradation of maltodextrin during oral processing, all subjects followed the same assessment protocol for the thickness evaluation for all stimuli. Consequently, the residence time in the mouth per stimulus was the same (~5s) for all subjects for all stimuli, so that the potential effect of α-amylase on thickness perception was standardized in the study. Subjects were not screened for their α-amylase activity since the purpose of the study was to determine the Weber fraction for thickness perception of untrained subjects of a model beverage in a broad range of viscosities rather than focusing on differences in thickness

perception between subjects varying in α-amylase activity. Between the tasting of the pairs, the panelists were asked to rinse their mouth with water and eat a piece of cracker to cleanse their palate.

2.3 Data Analysis

The percentages of stimuli assessed as "thicker than" the reference stimulus were determined for all stimuli pairs (2-AFC's) and transformed into z-coordinates to linearize the sigmoid psychometric function (Lawless and Heymann 2010). The data distribution was positively skewed. To normalize the data, the viscosity values were transformed into a natural logarithm function. The z-coordinates were determined as a function of the natural logarithm of viscosity and linear regressions were performed for each reference stimulus (McBride 1983; Le Berre 2008) (**figure 7.1B**). Using the equations resulting from the linear regressions, the JND can be calculated using the following equation:

$$JND = \frac{viscosity\ at\ 75\% - viscosity\ at\ 25\%}{2}$$

The values of JND's were then used to determine the Weber fraction (K) for thickness perception. K is defined as:

$K = \frac{\mathrm{JND}}{I}$ (Lawless and Heymann 2010)

where JND is the change in the physical stimulus (here: viscosity difference between stimuli) that was required to notice the discriminable difference and I is the concentration of the reference stimulus (here: viscosity of reference stimulus). The Weber fraction (K) for oral thickness perception of model stimuli ranging in viscosity from 10 to 100mPa.s was obtained by determining the slope of the plot of the JND's against the viscosity of the reference stimulus.

3. Results and Discussion

In **figure 7.1 A** the percentages of stimuli assessed thicker than the reference stimulus are shown as a function of viscosity of the reference stimulus for the five reference stimuli. **Figure 7.1 B** depicts the corresponding z-coordinates of the percentages of stimuli assessed thicker than the reference stimulus as a function of the natural logarithm of the stimulus' viscosity.

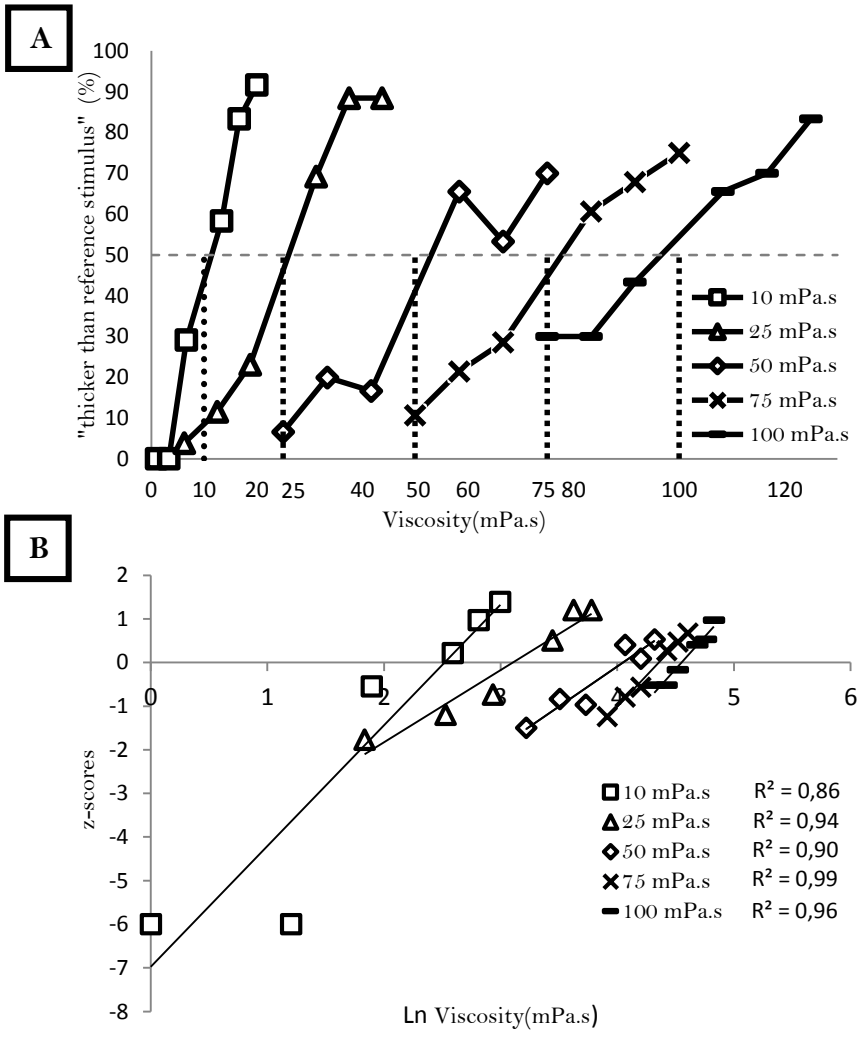


Figure 7.1. A. Percentages of selections of "thicker than reference stimulus" as a function of stimulus viscosity. **B**. Linear regression between z-coordinates as a function of the natural logarithm of viscosity. Each line shows the results between a set of reference and comparison stimuli.

Figure 7.1 A shows psychophysical functions for thickness perception of the liquid stimuli derived from the method of constant stimuli (Lawless and Heymann 2010). Typical sigmoid psychophysical functions are obtained for the reference stimuli with viscosities of 10, 25, 75 and 100 mPa.s. Small deviations from the typically expected sigmoid curve shape are observed for the psychophysical function corresponding to the constant reference stimulus with a viscosity of 50 mPa.s. This psychophysical function was gathered in the first sensory session. This might be the cause for the small deviations observed as the subjects might have been not familiarized sufficiently with the method of constant stimuli. We emphasize that the value of the Weber fraction (K) for oral thickness perception of model stimuli ranging in viscosity from 10 to 100mPa.s as described in the following was not considerably affected by the data of the reference stimulus 50 mPa.s.

The transformation of the psychophysical functions into z-coordinates to linearize the sigmoid functions (**figure 7.1 B**) were used to calculate the JND's (**figure 7.2**) and the corresponding Weber fractions (K) for each reference stimulus (**table 7.2**).

Table 7.2. Weber fractions (K) for oral thickness perception of five reference stimuli varying in viscosity.

Viscosity of reference stimulus (mPa.s)	K
10	0.31
25	0.37
50	0.43
75	0.25
100	0.22

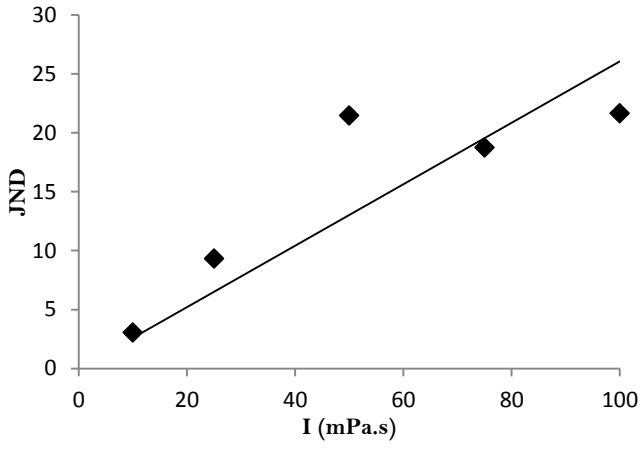


Figure 7.2. Linear relationship between Just Noticeable Difference of oral thickness perception (JND) with viscosity of the reference stimulus (I).

An increase of the JND with increasing viscosity of the reference stimulus is observed (**figure 7.2**). The JND determined with the 50 mPa.s reference seems to be higher than expected. As discussed previously, the first sensory session consisted of the comparison of the 50 mPa.s reference stimulus with its six comparison stimuli. The panel might have had more difficulties on discerning between the stimuli presented in the first session compared with following sessions since the subject might have been not familiarized sufficiently with the method of constant stimuli. Consequently, the JND and K for the reference stimulus with a viscosity of 50 mPa.s reflects a higher value corresponding to a lower sensitivity of the panel towards the comparison of the different viscosities. The value of the Weber fraction (K) for oral thickness perception of model stimuli ranging in viscosity from 10 to 100mPa.s was not considerably affected by the JND of the 50 mPa.s reference stimulus. The difference in the obtained Weber fraction (K) when all five reference stimuli (10, 25, 50, 75, 100mPa.s) were used compared with the obtained Weber fraction (K) when only four reference stimuli (10, 25, 75, 100mPa.s; data not shown) were used was $\Delta K=0.03$. We consider this difference negligible given the accuracy of the method of constant stimuli. We point out that this variation is smaller than the variation observed between

different studies reporting Weber fractions for the same taste modality (Schutz and Pilgrim (1957) , McBride (1983)).

The relationship between the difference in viscosity between constant reference and comparison stimulus (JND) and the viscosity of the reference stimulus (I) is illustrated in **figure 7.2**. From **figure 7.2**, a Weber fraction (K) for the five references stimuli ranging in viscosity from 10 to 100 mPa.s is obtained: K=0.26. The viscosity range of the five reference stimuli studied here covers the viscosity range of many beverages from low viscous beverages such as milk and juices to more viscous beverages such as drinking yoghurt, buttermilk and smoothies. The Weber fraction for thickness perception reported in this study (K=0.26) indicates that humans' sensitivity for thickness perception is comparable with humans' sensitivity for some taste modalities, for creaminess perception and for perception of firmness and spreadability assessed by hand. Schutz and Pilgrim (1957) studied the perception of sweetness of sucrose solutions and obtained a Weber fraction of K=0.17 using the method of single stimuli (Schutz and Pilgrim 1957). McBride (1983) reported a Weber fraction of K=0.13 for sucrose using the method of constant stimuli (McBride 1983). Furthermore, Schutz and Pilgrim (1957) studied the differential sensitivity for saltiness, sourness and bitterness. The Weber fractions for saltiness, sourness and bitterness obtained were K=0.15, 0.22 and 0.30, respectively (Schutz and Pilgrim 1957). The Weber fractions reported for sourness and bitterness are comparable with the Weber fractions for thickness perception reported in this study.

Stone and Bosley (1965) studied the olfactory discrimination sensitivity. They reported a Weber fraction of K=0.28 for the odors of acetic and propionic acid (Stone and Bosley 1965). Stone (1961) reported a Weber fraction of K=0.23 for the odor of 2-heptanone (Stone 1961). Slotnick and Ptak (1977) found a Weber fraction of K=0.32 for the odor of amyl acetate (Slotnick and Ptak 1977). Le Berre (2008) studied the importance of odorant proportions within mixtures. For this purpose, an experiment was conducted where the JND's of each odorant were determined. The obtained JND's correspond to Weber fractions of K=0.14 for

ethyl isobutyrate (strawberry odor), K=0.31 for allyl-α-ionine (caramel odor) and K=0.67 for ethylmaltol (violet odor) (Le Berre *et al.*, 2008). These studies indicate that the Weber fractions for olfaction are higher than the Weber fraction for oral thickness perception determined in this study and the Weber fraction for basic tastes. Furthermore, the K for olfaction seems to be dependent on the aroma component studied.

A recent study reported Weber fractions for creaminess perception when the apparent viscosity of dairy-based emulsions was varied by either modifying fat content or locust bean gum content. When the emulsions apparent viscosity was varied by modifying fat content the Weber fraction for creaminess was K=0.16. When the emulsions apparent viscosity was varied by modifying locust bean gum content the Weber fraction for creaminess was K=0.22 (Zahn *et al.*, 2013). Creaminess is a complex sensory attribute to which fat-related and viscosity-related perceptions contribute (Akhtar *et al.*, 2005). Zahn *et al.*, (2013) used stimuli with a more complex composition than the stimuli used in our study, which explains the use of an attribute that incorporates several dimensions. When the emulsions' viscosity was modified by changing the thickener content, the Weber fraction reported for creaminess is similar to the Weber fraction for oral thickness perception reported in our study.

Rohm and Raaber (1992) asked subjects to evaluate firmness and spreadability of edible fats by cutting the stimuli (spreads) and subsequently spreading them with a knife. Weber fractions of K=0.20 for firmness and K=0.27 for spreadability were determined (Rohm and Raaber 1992). These Weber fractions were obtained assessing food textural attributes non-orally. The Weber fractions are similar to the Weber fraction obtained in our study for oral thickness perception (K=0.26). This indicates that the sensitivity for food texture perception might be similar across different human sensory systems. An advantage of studying JND's of texture attributes of foods when assessing non-orally is that dumping effects are minimized, since taste and flavor cannot be assessed. In the case of our study,

maltodextrin stimuli were used since maltodextrin solutions display Newtonian flow behavior. However, the different concentrations of maltodextrin might have contributed to minor taste or flavor differences between stimuli. We attempted to minimize taste and flavor differences between stimuli differing in maltodextrin concentration by adding vanilla aroma to all stimuli to mask taste differences. We cannot exclude that the stimuli differed slightly in taste or flavor. The concentration of vanilla aroma for each stimulus was chosen based on a sensory pre-test by three subjects to determine the vanilla aroma concentration so that stimuli varying in maltodextrin concentration were perceived as iso-intensive with respect to sweetness and vanilla flavor. We emphasize that the difference in thickness between stimuli was described by all subjects as the most obvious and most dominant perceptual difference between stimuli. It should be noted that the range of maltodextrin concentrations of the comparison stimuli of the 50, 75 and 100 mPa.s reference stimuli was fairly narrow (26.0-33.3 % (w/w) for 50 mPa.s, 31.0-35.0 % (w/w) for 75 mPa.s and 33.3-36.0 % (w/w) for 100 mPa.s) suggesting that potential differences in taste and flavor between stimuli are expected to be small. Nevertheless, strictly speaking we cannot exclude that dumping effects occurred. Further, salivary α-amylase could have played a role on the thickness assessment of maltodextrin samples. It is known that α-amylase hydrolyses starch-based products leading to a decrease in viscosity (Hanson *et al.*, 2012). In the case of our study, we consider this effect negligible since the oral processing time of the stimuli was too short (less than 5 s) for a considerable effect of α-amylase on the degradation of maltodextrin to occur (de Wijk *et al.*, 2004).

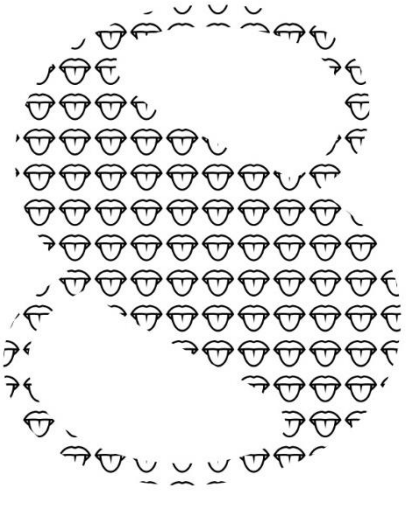
This emphasizes the challenges in studying JND's of texture properties of foods, since a variation in one texture attribute is often accompanied by a variation in another texture attribute such as taste or flavor. To minimize those effects as much as possible, we chose as stimuli model beverages with Newtonian flow behavior and fairly neutral taste with little flavor.

The Weber fraction (K=0.26) for thickness perception of Newtonian model stimuli (maltodextrin solutions) ranging in viscosity from 10 to 100 mPa.s was determined using the method of constant stimuli. The Weber fraction for oral thickness perception is comparable to Weber fractions reported in literature for perception of non-oral food firmness and spreadability and oral creaminess perception. The Weber fractions for olfaction reported in literature seemed to be higher than the Weber fraction for oral thickness perception and basic tastes, although seem to differ greatly depending on the aroma component. This demonstrates that the human sensitivity towards oral discrimination of viscosity of liquid stimuli is comparable to the human sensitivity towards specific texture and taste stimuli.

Abbreviations Used:

JND: Just Noticeable Differences, K: Weber fraction, I: Viscosity of reference stimulus

General Discussion



The aim of this thesis was to determine and characterize factors influencing oral coatings and their sensory perception. For this purpose, reliable methods to quantify oil and protein deposited on the tongue had to be developed to later study the macronutrients deposition. Further, the influence of stimulus properties on the formation and clearance dynamics of oral coatings and their impact on sensory perception were investigated.

This final chapter discusses the main findings of the study (summarized in **table 8.1)**, methodological considerations and provide an outlook for future studies.

Figure 8.1 is a tuning of **figure 1.1**, (Chapter1 - general introduction) with the knowledge acquired during this thesis included. As in chapter 1 (General Introduction), **figure 8.1** will guide the reader throughout the discussion.

Table 8.1. Main findings of this thesis.

Subject	Finding	Chapter
Formation dynamics of oral coatings	Oral coatings formed by stable o/w emulsions consist of individual oil droplets deposited on the tongue	**2**
	Oil fraction deposited on the back part of the anterior tongue was by a factor of ~1.5-2 times higher than oil fraction deposited on the front part of the anterior tongue	**2, 3**
	Oil/fat coatings are formed within the first 3 seconds of oral processing of liquid and semi-solids foods. Longer oral processing times do not increase oil deposition on the tongue.	**4, 5**
	Protein coatings are formed within the first 3 seconds of oral processing of liquid foods	**6**

Subject	Finding	Chapter
Properties of oral coatings	Oil fraction deposited on tongue increased linearly with oil content of o/w emulsion	***2***
	Protein deposition on tongue increased with protein content of the liquid stimulus	***6***
	Fat fraction deposited on tongue is higher in gels with unbound droplets compared to gels with bound droplets	***5***
	Oil and protein deposition on tongue immediately after expectoration of stimuli was not affected by formation of flocs due to saliva-protein interactions	***3,6***
	Increasing protein content, lead to a negative relation on the oil fraction created by lysozyme stabilised emulsions, but had no relation on the oil fraction created by Na-caseinate stabilised emulsion.	***3***
	Presence of thickener decreased oil and protein deposition on tongue	***3,6***
Clearance dynamics of oral coatings	Clearance of oil and fat deposits from the tongue occurs within the first 45 seconds after expectoration and is hence slower than formation dynamics of oral coatings	***2, 3, 5***
	Protein type influenced clearance dynamics of oil coatings with Na-Caseinate stabilised emulsions being faster cleared from the tongue than Lysozyme stabilised emulsions	***3***

Subject	Finding	Chapter
Sensory perception of oral coatings	After-feel perception of o/w emulsions followed a semi-logarithmic relationship with oil fraction deposited on the tongue (Weber-Fechner's law)	**2**
	Fat related attributes of oil coatings of lysozyme stabilized o/w emulsions were perceived as less intense compared to oil coatings from Na-caseinate stabilized o/w emulsions	**3**
	Fatty perception is higher in coatings from gels with unbound droplets compared to gels with bound droplets	**5**
Effect of oral coatings on subsequent taste perception	Oil fraction deposited on the tongue did not significantly affect subsequent sweetness perception of sucrose solutions. Amount of oil droplets deposited on the tongue was insufficient to form a hydrophobic barrier to reduce the migration of hydrophilic tastants to the taste buds	**4**
Thickness perception	Oral thickness sensitivity is comparable to kinaesthetic thickness sensitivity	**7**

Figure 8.1 answers the research questions which were raised in **figure 1.1**. in Chapter 1 – General Introduction.

In **figure 8.1** the stimuli properties studied in this thesis are specified for each oral coating: protein coating and oil coating. Further, four symbols are used to characterize the effects on the oral coatings: formation of the coating, clearance of the coating, effect on mouth-feel of the coating and finally, effect on after-feel of the coating.

When an effect on the coating was shown to occur due to a specific property of the stimulus, the symbol representing the effect on the coating is located in front of the stimulus property.

For instance, modifying the protein content on the aqueous phase of the o/w emulsion had a positive effect on the formation of the protein coating and on the mouth-feel.

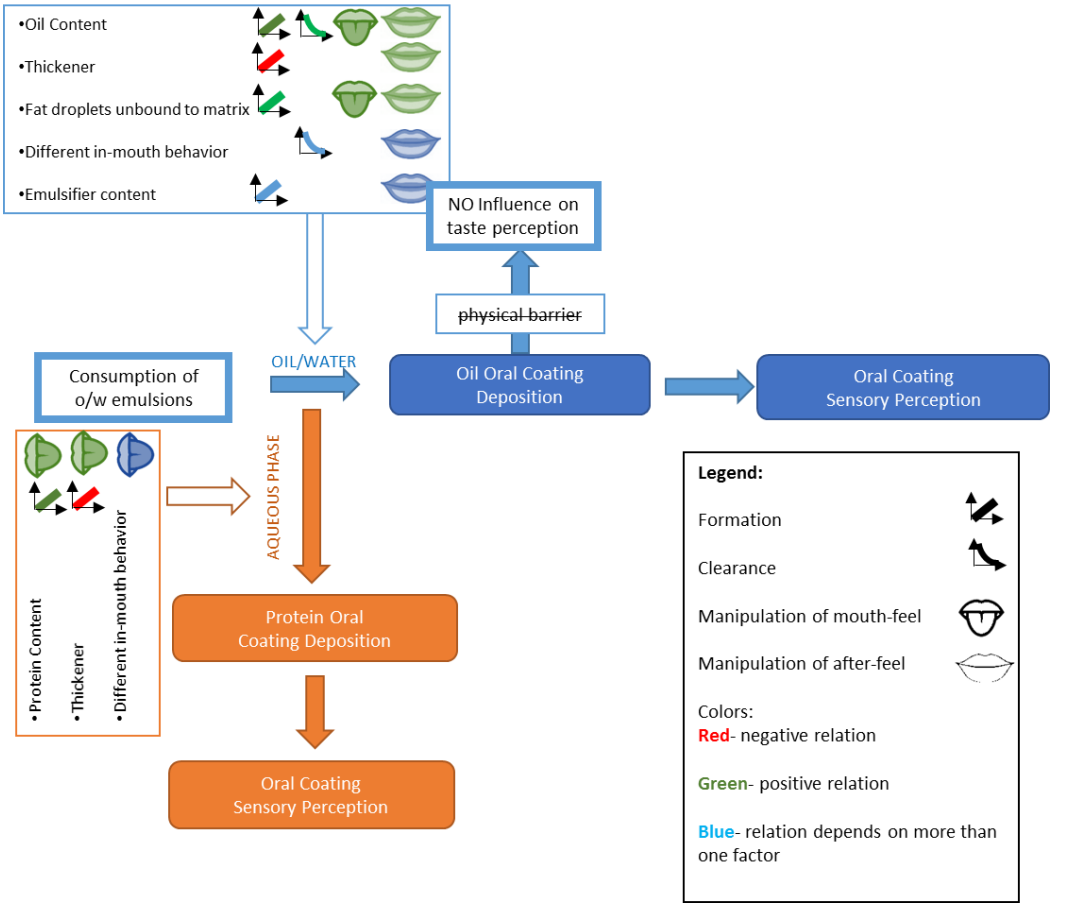


Figure 8.1. Outcome of the main results from this thesis. Samples processed on the study of oil coatings consisted on o/w emulsions. Samples processed on the study of protein coatings consisted on protein aqueous samples.

8.1 Methodologies to Quantify Oral Coatings

The study of oil oral coatings was performed by *in vivo* fluorescence. *In vivo* fluorescence provides a direct measure of oil oral coatings without damaging any of its components. Further, it allows the study of the coating on different parts of the tongue. This is an advantage to understand how the coating is deposited, and how the morphology of the tongue affects the deposition of the coating. Results on chapter 2, showed that *in vivo* fluorescence measurements was a reliable method to quantify oil fraction (mass of oil/ area tongue) deposited on the tongue. Furthermore, as this is a relatively easy method to apply, the sensory profiling of the coatings could be drawn simultaneously as the fluorescence measurements. Nevertheless, the fact that the probe to measure the different parts of the tongue is handled by humans could be a limitation. Possible human interferences on handling the probe, *i.e.* positioning the probe in slightly different places of the tongue depending on the subject, is not completely excluded. We have tried to minimize this interference by having at least 15 subjects participating in our studies, and by making triplicates of each measurement. Further, to reach accurate results on the quantification of oil deposited on the tongue by *in vivo* fluorescence, a new calibration was made for each sample used in the study. This is needed due to the interference of the fluorescence of curcumin which might be different for different ingredients in the samples. As such it is of extreme importance to find the correct fluorescence dye for the macronutrient that is aimed to be studied.

Protein coatings were also attempted to be studied by *in vivo* fluorescence, but the pursuit for a precise and accurate protein dye was found to be fruitless. As such, another method had to be developed to study protein coatings.The study of protein coatings was made *ex vivo*. Firstly the coating was collected by cotton swabs, and subsequently the protein was quantified by the Lowry method. It is possible that the oral coating was not completely collected by the cotton swabs. We have overcome this limitation by quantifying the protein coating in terms of

protein concentration. By weighting the amount of coating taken out of the tongue with the swabs, we were able to treat protein coatings in terms of mass protein/mas coating and thus be able to compare different properties of the processed samples at the same level.

Similarities in behavior of oil and protein coatings dynamics were observed and will be discussed in the next sub-chapters. Nevertheless, it is important to notice that oil coatings and protein coatings were quantified on distinct studies. Therefore, future studies could consider studying these two macronutrients with the same method and the same participants. Nevertheless, we believe that the main conclusions will remain unchanged.

8.2 Properties Influencing Deposition of Oral coatings

8.2.1 Formation Dynamics of Oral Coatings

Oil oral coatings were first studied in literature after consumption of bulk oil. Pivk *et al.*, (2008a,b) chose "thickness" of lipid layer as the physical parameter to describe the coatings. Thickness suggests that a continuous film of oil is deposited on the tongue. *In vitro* experiments (chapter 2), analysed the microstructure of the o/w emulsions on the surface of pig's tongue, using Confocal Laser Scanning Microscopy. Images of pig´s tongues were taken under two conditions: with and without human saliva. For each condition 3 images were made: (i) plain tongue, (ii) tongue with 20% (w/w) o/w emulsion stabilised with 3% (w/w) Na-caseinate, (iii) tongue (ii) *i.e.* with the o/w emulsion, after being rubbed with a fresh piece of tongue (to simulate the rubbing of the human tongue against the palate). The structure of the 20% (w/w) o/w emulsion did not change under the mimicked in-mouth conditions. Hence, stable o/w emulsions are likely to be stable under in mouth conditions. Therefore, the oral coating formed by the emulsions is not a homogeneous layer of oil but consists of oil droplets. Thus, based on chapter 2 it is recommended to quantify oral coatings by oil fraction deposited on the tongue

(mass of oil per surface area of the tongue) rather than oral coating thickness as it has been often used in literature.

Adams *et al.*, (2007) reported a technique for oral coatings visualization with the use of an *in-vivo* fluorescence technique. This apparatus allowed the gathering of real-time images in the mucosal surfaces from around the oral cavity. The technique allowed to visualize the in-mouth behavior of corn and castor oil. Emulsification of the oils due to saliva was observed, with lower viscosity samples creating smaller oil droplets (Adams *et al.*, 2007). This demonstrates that oral processing of bulk oils creates emulsified oil droplets adhered to the mucosa. This finding further supports the recommendation to use oil fraction as the physical parameter to describe oil coatings formed by o/w emulsions and for bulk oil rather than oil coating thickness.

Oil fraction deposited on the back part of the anterior tongue was found to be higher (~1.5-2 folds) for all the emulsions studied (chapter 2 and 3) compared to the front part of the anterior tongue, independently of the ingredients type and/or content present in the emulsion. The most important papillae for the formation of the oral coating are the filiform papillae, which are more predominant at the back part of the anterior tongue (**figure 1.2**). These papillae cause the roughness of the back part of the anterior tongue surface. In contrast, the front part of the anterior tongue consists mainly of fungiform papillae. This leads to a smoother surface of the front part of the anterior tongue in comparison to the back part of the anterior tongue (Kawasaki, *et al.*, 2012). It is likely that oil droplets are easier mechanically entrapped between the filiform papilla in the back part of the tongue. Further, the movement of the tongue could also play an important role on the spatial variation of oil deposition. When swallowing, the back part of the anterior tongue rubs less against the teeth and palate (ChiFishman *et al.*, 1996, Pouderoux *et al.*, 1995) which likely creates less degradation of the oil oral coating compared to the front. In a taste perception view this is not a satisfactory result. The back part of the tongue covered with filiform papillae, where oil fraction was found to be higher, do not contain taste

buds. The front part of the tongue with fungiform papillae, where oil fraction was found to be lower, contain taste buds and thus taste perception occurs. As such, taking into account the composition of the orally processed sample, it is likely that sensory perception of the coating is not able to reach its ideal maximum.

Formation of oil/fat coatings was studied in chapter 4 for liquid emulsions, and in chapter 5 for emulsion-filled gelatine gels. For both systems the coatings were formed within the first 3s of oral processing. For the case of the liquid emulsions, the fast saturation of oil deposited on the tongue surface is in accordance with the suggestion that o/w emulsions contain oil droplets easily available which can rapidly adhere to the tongue surface. This suggests that within the natural drinking time of one sip (20mL) of a liquid stimulus (3.3±0.2 s for full fat milk), the tongue surface is already coated with the beverage´s oil which adheres to the tongue. In the case of the emulsion-filled gels, it is important to note that gelatine was used as solid matrix. Gelatine melts at body temperature. At short oral processing times (~2s) it is unlikely that gelatine reached body temperature, and thus did not completely melt. As such, the effect of mastication is probably more important for the oil droplets release compared to the melting of gelatine. This suggests that the first bites are the most relevant for the formation of fat coatings on the tongue. Devezeaux *et al.*, 2014 showed that for emulsion-filled gels, sensory attributes related to first bite include sticky and elasticity. Important sensory attributes such as creaminess arise at a longer stage of mastication (Devezeaux.*et al.*, 2014). This fact may be due to the accumulation of fat coatings in mouth from the first bites together with the contribution of other ingredients such as the melting of gelatine, which may occur at longer oral processing durations.

Chapter 6 studied the deposition of the protein in the oral coating after consumption of 3% (w/w) Na-caseinate, 6% (w/w) Na-caseinate, 3% (w/w) Na-caseinate with 0.2% (w/w) xanthan gum and 3% (w/w) lysozyme. As in oil deposition, protein deposition on the tongue was fast, with the maximum being reached after an oral processing time of 3s for 3 of the 4 orally processed samples

(the forth sample - 6% (w/w) Na-caseinate, reached the maximum deposition at 9s). de Jongh *et al.* (2007), found that swirling dressings for longer times in the mouth (5s *vs.* 20s) created higher deposition of protein/carbohydrates on the middle of the tongue. The difference between our results and others are likely due to the readily availability of the macronutrients in the liquid solutions used in our study, which are more easily in contact with the tongue papillae facilitating their deposition on the tongue in contrast to the soft semi-solids used by de Jongh *et al* (2007). As mentioned before, this suggests that within the natural drinking time of one sip (20mL) of a liquid stimulus the tongue surface is fully coated with the macronutrients present in the beverage. This indicates that the coating perception from these beverages is likely to reach its maximum, at a natural drinking time.

8.2.2 Properties of Oral Coatings

Chapter 2 showed that oil fraction deposited on the tongue surface immediately after stimulus expectoration increased linearly with increasing oil content of the o/w emulsion. Other studies have shown a logarithmic increase when bulk oil is consumed at higher volumes (Pivk *et al*, 1998b). A logarithmic increase of oil deposited on tongue with volume of bulk oil orally processed suggests that saturation of oil deposited on the tongue occurs when stimuli with high oil volume are consumed. In contrast, our results show that when oil content is lower than in bulk oils, *i.e.*, oil content ranging from 1-20%, as often occurring in commercial foods and beverages, saturation of oil deposited on the tongue does not occur. Chapter 4, studied the formation of oil oral coatings when different oil content of o/w emulsions were orally processed. Interesting to note is that longer oral processing did not increase significantly the oil fraction on the tongue for emulsions with the same oil content. But, higher oil content on the o/w emulsion creates higher oil fraction. This suggests that the availability of the oil droplets in the emulsion is more relevant for the oil deposition than the amount of time which the sample is processed in mouth.

Fat coatings created by emulsion-filled gelatine gels were studied in chapter 5. Once more, it was seen that with higher fat content in the processed sample, higher fat was deposited on the tongue. Further, the interactions between the fat droplets and the matrix of the gelatine gel influenced the fat coating. Fat droplets unbound to the matrix (Tween 20 as emulsifier) created higher fat deposition compared to fat droplets bound to the matrix (WPI as emulsifier). During oral processing, emulsion-filled gels undergo breakdown into smaller fragments, when the fat droplets are unbound to the matrix these are easily released into the oral cavity and have a higher availability to deposit on the tongue. On the other side, fat droplets bound to the gelatine matrix are less available to deposit on the tongue as they are likely still surrounded by the gelatine matrix.

Protein oral coatings have a similar formation dynamics as oil oral coatings (chapter 6). Protein content of the stimulus had an effect on the protein concentration of the oral coatings, *i.e.*, higher protein content of the stimulus led to higher protein concentration in the oral coatings. This is thought to be due to the availability of the protein on the processed sample, with samples with higher content having more protein which can be deposited on the tongue.

Studies on oil/fat oral coatings (chapter 2, 3, 4 and 5) and on protein oral coatings (chapter 6) suggest several parallels on the deposition dynamics of both macronutrients.

According to the results of this thesis, the underlying mechanisms for the adherence of an ingredient to the tongue surface are based mainly on mechanical forces. It is possible that under mouth-conditions, the shear of tongue against the palate has a stronger influence on adhesion and spreading compared to chemical-physical forces. Our results contrast with *ex vivo* studies which suggest the importance of electrostatic interaction between oil droplets and protein with surfaces mimicking oral conditions. Dresselhuis *et al.*, (2008a) showed that the tongue is hydrophobic when it is dry, but hydrophilic when covered with a layer of saliva. It was hypothesized that oil coatings behave differently compared to protein coatings due to differences in hydrophobicity. Malone *et al.*, (2003) studied

the interfacial interactions between emulsions stabilized either with a non-ionic emulsifier (Tween 60) or a positively charged emulsifier (chitosan) on a mucin film (with negative charge) using evanescent wave spectroscopy. Results showed that oil droplets stabilized with chitosan were adsorbed onto the mucin film, while oil droplets stabilized with Tween 60 did not show any adsorption. This was suggested to be due to electrostatic interactions between the oil droplets stabilized with chitosan and the mucin film. Dresselhuis *et al.*, (2008b), identified the importance of not only the adhesion of the sample onto the surface, but also the facility of the samples spreading on the surface for sensory perception. Dresselhuis *et al.*, (2008b) studied the effect of electrostatic, steric and hydrophobic forces between protein stabilized o/w emulsions and hydrophobic and hydrophilic glass surfaces, using a flow cell with light microscopy. The adhesion and spreading of the emulsion droplets was enhanced when the electrostatic and steric forces were reduced and hydrophobic attraction between droplets and the glass surface occurred. It seems clear that the adhesion of a sample onto a solid surface (*ex vivo*) is dependent on several physical and chemical forces. Comparisons with previous results and the results of our thesis confirm the importance of conducting experiments under *in vivo* conditions.

The behavior of o/w emulsions in mouth has been previously suggested to affect oral coatings (Vingerhoeds *et al.*, 2009). One of the major contributors to different behavior of emulsions under in-mouth conditions are the interactions with saliva. Flocculation of o/w emulsions can occur when emulsions are mixed with saliva. Whether the flocculation is reversible or not depends on the surface charge of the emulsion droplet, pH, salts and composition of salivary biopolymers (Silletti *et al.*, 2007). In our study, we focused on studying flocculation through changes of the surface charge of emulsion droplets.

Salivary biopolymers are negatively charged at neutral pH. Emulsion droplets stabilized with highly negative charged proteins do not flocculate with salivary biopolymers, while weakly negative charged to neutral emulsion droplets experience reversible flocculation upon mixing with salivary biopolymers.

Positively surface charged emulsion droplets flocculate irreversibly with negative biopolymers in saliva due to bridging interactions (Silletti *et al.*, 2007). Chapter 3 studied the effect of protein emulsifier type by investigating two types of emulsions: lysozyme stabilised emulsions, which form irreversible agglomerates when mixing with saliva, and Na-caseinate stabilised emulsions which do not form agglomerates with saliva. When comparing oil fraction deposited on the tongue, it was seen that at a same protein content (3% (w/w)), oil coatings formed by Na-caseinate stabilised emulsions did not differ significantly from oil coatings formed by lysozyme stabilised emulsions. Chapter 6 studied protein coatings and once more the different type of proteins (lysozyme *vs.* Na-caseinate) were investigated due to their different in-mouth behavior. Results showed that lysozyme and Na-caseinate solutions formed similar oral protein deposition.

When increasing the protein content of the o/w emulsions (chapter 3), lysozyme stabilised emulsions created less oil deposition while oil coatings from Na-caseinate stabilised emulsions were not affected. It is possible that with higher concentrations of unadsorbed lysozyme more and larger agglomerates are formed upon mixing with saliva in the oral cavity. The larger agglomerates might be easier expectorated than smaller oil droplets (as in the case of Na-caseinate stabilized o/w/ emulsions). As lysozyme forms aggregates, it is plausible that the aggregates were too large to enter the voids between the papillae during oral processing. The same occurred when xanthan gum was added to o/w emulsions or to an aqueous 3% Na-caseinate sample. It is likely that xanthan created a matrix which entraps the oil droplets and the proteins (chapter 3 and chapter 6), which also reduces the capability of entering the voids of the papillae.

This thesis demonstrates that the ability of oil droplets or protein to enter the voids between the papillae is a key factor which determines the amount of coating deposited on the tongue. This can be influenced by either adding a thickener which entraps the ingredients, or by changing the configuration of the oil droplets through aggregation (**figure 8.2**).

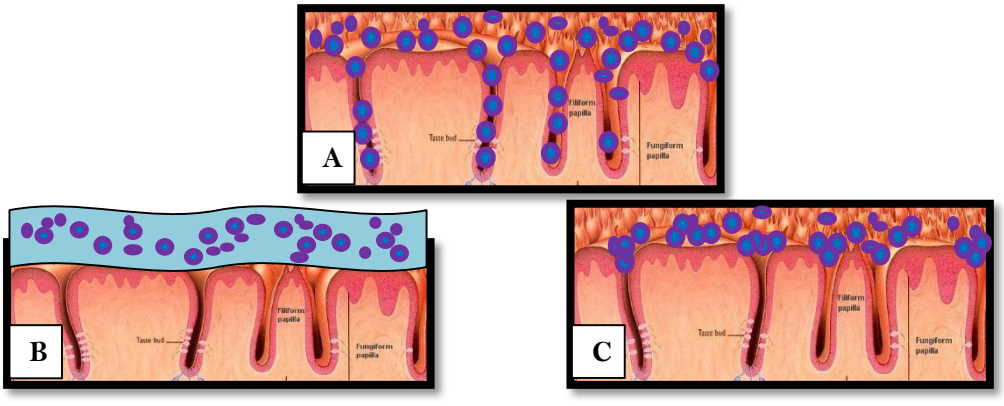


Figure 8.2. Different oral coatings (blue circles represent oil droplets). A- oil oral coating formed by a o/w emulsion. B- oil oral coating formed by a o/w emulsion with xanthan (layer on top represents the matrix of xanthan-gum entrapping the oil droplets). C- oil oral coatings formed by a o/w emulsion stabilised by lysozyme-aggregation.

8.2.3 Clearance Dynamics of Oral Coatings

Clearance of oil/fat coatings from the tongue was studied in chapter 2 and 3 for liquid emulsions, and in chapter 5 for emulsion-filled gelatine gels. Clearance of fat/oil from the tongue occurs due to three main effects: saliva flow (Adams *et al.*, 2007), movements of the tongue against the palate, which can damage the fat coating (Camacho *et al.*, 2014), and food particles that can disrupt the oral coating due to mechanical abrasion. Saliva flow can increase the speed of clearance of the coating through emulsification of the consumed sample (Adam *et al.*, 2007). Further, as discussed in chapter 8.1, while swallowing the front anterior part of the tongue rubs against the teeth and palate (ChiFishman *et al.*, 1996, Pouderoux *et al.*, 1995) which creates degradation of the oral coating. It is also suggested that food particles can disrupt the oral coating due to physical irregularities of the material which can rub against the coating and clear it from the oral mucosa.

In general, clearance of oil/fat coatings followed a similar tendency for all stimuli studied in this thesis. Most of the coating (> 60%) is cleared from the tongue in the first 45s. The exception to these findings are oral coatings formed by lysozyme stabilised emulsions, which have a slower oil clearance. Van Aken *et al.* (2007) reported that complex formation with salivary proteins and lysozyme can

take place in the saliva fluid as well as with the mucous layer. Complex formation with the mucous layer might slow down clearance. On the other hand, the coating formed by the remaining emulsions is likely to have no or very little binding with the mucous layer and as such, easier to be washed away by saliva and rubbing against palate.

8.3 Influence of Oral Coatings on Sensory Perception

8.3.1 Mouth-feel and After-feel of Oral Coatings

Perception of oil and protein oral coatings is highly influenced by the lubricating layer created on the tongue, for instance due to higher oil deposition or due to the presence of thickener on the processed sample. Chapter 2 showed a logarithmic relationship (Weber-Fechner law) between the fatty film and flavour intensity and oil deposition until 90s of the coating's residence time. Relationships between amount of oil coating and fatty film perception were reported previously. Pivk *et al.*, (2008b) found a linear correlation between thickness and fatty film and deposition of oil on the tongue. de Wijk *et al.*, (2009), used semi-solid custard desserts in order to analyse the clearance of oral coating using turbidity rinses. With this method, a linear correlation between the turbidity slopes for the first rinse and the individual fattiness slopes was observed. Differences between our results and others most likely lay on the different samples used, with different structures, and on the different methods used to quantify the coatings.

Chapter 5 studied the fatty mouthfeel and afterfeel perception of the coating formed by emulsion filled-gels. Perception of emulsion filled-gels followed the same trend as the deposition of fat from the gels: fat droplets bound to the gelatine matrix (stabilised with WPI) are less available to deposit on the tongue, as they are likely still surrounded by the gelatine matrix, and as such, the coatings formed by these emulsion-filled gels also had a lower fatty perception. Yet, as suggested on Chapter 3 and chapter 6, the perception of the coating does not only relate to the physical oil deposition on the tongue. The existence of a lubricating

layer created by a thickener decreases oil fraction on the tongue and protein deposition on the tongue, but influences the perception of positive attributes such as creaminess. Further, although lysozyme stabilised emulsions and Na-caseinate stabilised emulsions create similar oil depositions at t=0s after expectoration of the emulsion, the perception of coatings created by these two emulsions is very different. Lysozyme provokes an astringent and dry sensation in the mouth, which are sensations that are likely to reduce the intensity of attributes such as fatty film and creaminess. Vingerhoeds *et al.* (2009) suggested that the flocculation of lysozyme stabilized o/w emulsions had similarities to saliva flocculation observed by addition of tannins. The flocculation with proteins in saliva and mucous layer reduces the lubrication properties of saliva and increases friction in mouth which leads to astringent and rough after-feel. Nevertheless, when Na-caseinate was used as an emulsifier, fat related attributes were perceived as more intense when higher oil fraction was deposited on the tongue. This is likely due to the more neutral taste created by the Na-caseinate in comparison to the strong astringent and sweet effect of lysozyme.

8.3.2 Oral Coatings Influence on Subsequent Taste Perception

Chapter 4 focused on the study of the possible effect of oral coatings formed by o/w emulsions on the subsequent sweetness perception. Previous studies have hypothesized that a physical hydrophobic barrier formed by the fat coating on the tongue surface hinders the migration of tastants through the coating leading to less tastants molecules reaching the taste buds, thus leading to a decline in taste intensity (Ahn *et al.*, 2002, Madrigal-Galan *et al.*, 2006, Lynch *et al.*, 1993, Valentova *et al.*, 1998). To test this hypothesis, the effect of the oral coating on the tastant stimulus was analysed by coating the subjects' tongues with o/w emulsions and subsequently evaluating the sweetness intensity of sucrose solutions. The subjects were trained and capable to distinguish between sucrose stimuli differing by 0.3% in sucrose content (relative to a 4% sucrose solution). No significant influence of oil coatings deposited on the tongue surface on subsequent

sweetness perception of liquid sucrose solutions was observed. It is important to note that chapter 2 gave us an important visual knowledge on the oil coatings, showing individual oil droplets attached to the papillae instead of a homogeneous layer of oil. It is then clear, that the oil droplets deposited on the tongue do not form a hydrophobic barrier that is sufficient to reduce the accessibility of sucrose to the taste buds. This suggests that in the range of oil content studied (which is similar to realistic foods) the migration of sucrose molecules to the taste buds is not sufficiently hindered and consequently sweetness perception is not altered. Our results differ from previous studies on the same topic, as others studies used flavoured bulk oils to form oil coatings. Coatings stimuli reported in literature range from bulk sunflower oil, coconut oil to commercially available emulsions (such as milk and mayonnaise) (Lynch *et al.*, 1993, Valentova *et al.*, 1998). This difference in stimuli might have led to differences in oil coatings. It is likely that the oil coatings formed with bulk oil had a more homogeneous fat layer and might have created a thicker physical barrier and thus suppressed the migration of hydrophilic tastants to the taste buds. To evaluate the possible physical interference of oil coatings with subsequent taste perception, the coating should have as little taste and flavour as possible. It is known that commercially available emulsions have a complex food matrix and do not only contain fat, but also taste and flavour molecules. The ingredients from the oral coating might have contributed to the suppression of the sweet taste by various mechanisms, *e.g.* texture-taste, taste-taste or odor-taste interactions. Another reason might lay on taste substances or flavor molecules present in the oral cavity after the consumption of commercially available emulsions which could have masked the succeeding taste of sweet taste solutions.

Sucrose is a hydrophilic molecule and a large molecule. If sucrose can diffuse through the oil coating without any variation on taste sensitivity, it is suggested that other basic tastes will also not be influenced by the physical barrier caused by the oil coating. Taste differences found on other studies are likely due to texture-taste, taste-taste or odor-taste interactions.

8.4 Thickness Perception of Liquid Model Stimuli

The Weber fraction for thickness perception reported on chapter 7 indicates that humans' sensitivity for thickness perception is comparable with humans' sensitivity for some taste modalities, for creaminess perception and for perception of firmness and spreadability assessed by hand. As mentioned before, little is known on texture sensitivity. It would be desirable for food technologists to know more about sensitivity of other oral texture attributes such as slipperiness for *e.g.* custards, or melting for *e.g.* chocolate.

8.5 Main Conclusions

Oral coatings are formed within the first seconds in which beverages and semi-solids are in contact with the tongue. Oil coatings from stable o/w emulsions form individual oil droplets on the tongue.

Oil oral coatings formed by o/w emulsions are highly influenced by the oil droplets availability. In the case of consumption of emulsion-filled gelatine gels, the droplets interaction with the gelatine matrix has an effect on fat deposition on the tongue, with unbound droplets creating higher deposition compared to bound droplets.

Protein oral coatings have a similar formation dynamics as oil oral coatings. Further, several properties which influence oil coatings also influence protein coatings: samples with higher ingredient content create coatings with higher ingredient deposition on tongue, presence of thickener in the sample creates coatings with lower oil fraction and lower protein concentration. Finally, different in-mouth behavior of the orally processed samples (*i.e.*, aggregation with salivary biopolymers) does not have an influence on the deposition of the coatings, but oil droplets which form electrostatic interactions with the mucus layer from the tongue are more difficult to be cleared.

Clearance of the oil coatings is a fast process with around 60% of the oil being cleared from the tongue in the first 45 seconds after expectoration of the sample.

Perception of oil and protein oral coatings is highly influenced by the lubricating layer created on the tongue, for instance due to higher oil deposition or due to the presence of thickener on the processed sample. Protein behavior in mouth highly influences the perception of the coatings, with lysozyme having a high astringency intensity likely due to the formation of aggregates with the saliva.
Oil oral coatings from o/w emulsions with neutral taste were found to not significantly influence subsequent sweetness perception of liquid sucrose solutions. This is likely due to insufficient oil droplets deposition on the tongue to hinder the accessibility of sucrose to the taste buds.

8.6 Methodological Considerations

In this thesis, model foods were chosen to study oral coatings. Model foods were chosen as it is easy to control a range of parameters from ingredients composition and content to, for instance, pH. This allows to pin point specifically which property of the stimuli creates a specific behavior. For this thesis a range of model foods, from semi-solids to high viscous o/w emulsions to low viscous o/w emulsions, was designed. Emulsion-filled gels are very common models for cheeses and sausages. The liquid o/w emulsions had a range of viscosity, oil content and protein content representative of beverages from thick liquid yoghurts to low fat milk. Although the design of the stimuli was representative of a great number of real foods, there is no denial that real food have a much more complex matrix compared to our stimuli. As such, research on oral coatings of real food should be considered and compared with our results.
As mentioned in previous chapters, oil oral coatings were measured by *in vivo* fluorescence and protein coatings were measured by collecting the coating from the tongue with cotton swabs and quantification of protein by the Lowry method. Although these methods proved to be efficient and reproducible, none of the coatings´ measurements was made simultaneously on the quantification of oil and protein. It would be valuable to have a method which would quantify both macronutrients in the same measurement in order to study simultaneously the

development and clearance of the oral coatings. As such, trials with for example RAMAN spectroscopy could be made in order to verify the possibility of using this method for the mentioned purpose.

The results of oral coatings deposition contained a large variability, likely due to different individual morphologies of the tongue. In each study the subjects were chosen to be the most homogeneous possible, with similar age group and eating habits. Nevertheless, within the group different tongue morphologies were observed. It would be interesting to study this variability systematically, for instance within a nationality, or within groups of supertasters and non-tasters. Research on supertasters and non-tasters have shown that differences on taste sensitivity may lay on the differences on the morphology of the tip of the tongue. Super tasters are thought to have more density of fungiform papillae compared to non-tasters. One of the hypothesis stated in this thesis is that places on the tongue with higher density of papillae have more deposition of coatings. As such, a study comparing the oral coatings deposition of subjects, which the tongue´s tip had been characterized morphologically could add valuable knowledge to the field.

8.7 Future Recommendations

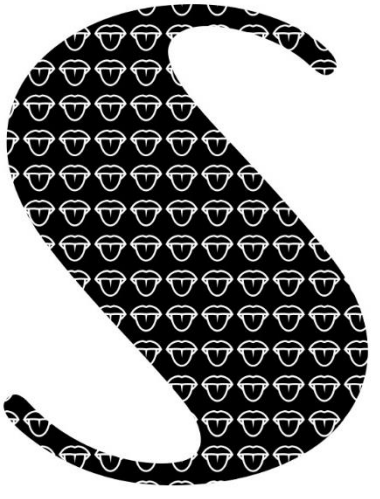
This thesis focused on the effect of oral coatings on the perception of after-feel, mouth-feel and taste. Methods for quantification of oral coating on the tongue were developed for that purpose. Nevertheless, it is important to not neglect the effect of aroma on the full sensation of eating. As such, an important future recommendation would be the development of techniques which would measure the oral coatings in the pharyngeal mucosa, where it is hypothesized that aroma molecules adhere.

As health problems are increasing due to high intake of sugars and salts, oral coatings made with these molecules should be studied in order to maximize the perception and minimize the addition of these molecules in food. First though, methods to describe the coatings of these molecules should be developed. Future research could explore the measurement of salty coatings through, for instance,

conductivity, and the measurement of sugars by, for instance, collecting the coating and measuring it through HPLC.

On chapter 4 it was discussed that the oil coating formed by a 20% o/w emulsion with neutral taste did not create a sufficient hydrophobic barrier to promote the decrease of subsequent taste. This result differs from the finding of several others which used different stimuli to coat the tongue. It would be interesting to know how much oil is needed in an o/w emulsion to create taste suppression. This knowledge would be valuable for food technologists mask undesirable tastes.

Lastly, in this thesis oral coatings created by model foods were studied. These model foods ranged from semi-solids (emulsion-filled gels) to high viscous liquids (o/w emulsions with thickeners added) to low viscous liquids. As mentioned in the previous sub-chapter, model foods are usually used in research as their properties are easily controllable. Nevertheless, there are significant differences between real foods and model foods. As such, it would be interesting to study oral coatings of real food and beverages.



UMMARY

Oral coatings are residues of food and beverages that coat the oral mucosa after consumption. Several studies have reported on the lubrication properties in mouth, and the after-feel and after-taste impact of oral coatings. Further, oral coatings have been suggested to influence subsequent taste perception. Although it is well known that oral coatings can influence sensory perception, there was little information available on the chemical composition and physical properties of oral coatings. As such, the aim of this thesis was to understand which factors influence the composition of oral coatings and their sensory perception.

This study started with the development of an appropriate calibration method for an already described methodology to quantify oil oral coatings: in vivo fluorescence. Further, the samples studied were shifted from pure oil (used on previous studies) to a more realistic food beverage: o/w emulsions. Pig´s tongues are known to be a good model of human tongue. As such, Chapter 2 used pig´s tongues on the calibration of the method, to mimic the fluorescence in mouth of oil coatings. On chapter 2, Confocal Scanning Laser Microscopy images showed that stable o/w emulsions (1-20% (w/w)) stabilised by Na-caseinate created individual oil droplets on the surface of the pigs tongue, as such a new descriptor for oil coatings was developed. Oil fraction, *i.e.* mass of oil per surface area of the tongue, was shown to be higher on the back compared to the front anterior part of the tongue. This is thought to be due to the morphology of the tongue and abrasion of the oil coating owed to the rubbing with the palate. Further, *in vivo* measurements showed that oil fraction deposited on the tongue increased linearly with oil content of o/w emulsions. Coating clearance from the tongue was a fast process with around 60% of the oil being removed on the first 45s. After-feel perception (Fatty Film and Flavour Intensity) was shown to be semi-logarithmic related to oil fraction on the tongue.

Chapter 3, further investigated different properties of 10% (w/w) o/w emulsions that influence the oil fraction deposited on the tongue, its clearance and after-feel perception. Three different properties were studied: protein type, protein content and viscosity of the o/w emulsions. To study the influence of protein type, two

different proteins which behave differently in-mouth were studied: Na-caseinate - creates emulsions which do not flocculate under in mouth conditions, and lysozyme – creates emulsions which flocculate under in mouth conditions. To study the influence of protein content, three concentrations of Na-caseinate and lysozyme were used (0.2, 3, 5.8% (w/w) all in excess to stabilize the water/oil interface). To study the influence of viscosity of o/w emulsions, three o/w emulsions stabilized with 3% (w/w) Na-caseinate were thickened with varying concentrations of xanthan gum (0-0.5%) (w/w).

Generally, the irreversible flocculation of lysozyme stabilized emulsions with saliva did not create a significant difference on oil deposition compared to emulsions stabilized with Na-caseinate, immediately after expectoration of the emulsions. Nevertheless, lysozyme stabilised emulsions caused slower oil clearance from the tongue surface compared to emulsions stabilized with Na-caseinate. Protein content had a negative relation with oil fraction on the tongue for lysozyme stabilized emulsions and no relation for Na-caseinate stabilized emulsions. The presence of thickener decreased deposition of oil on tongue, although viscosity differences (*i.e.*, thickener content) did not affect oil fraction. After-feel perception of creaminess and fatty-film was strongly influenced by the presence of thickener likely due to lubrication in-mouth, *i.e.*, the higher the concentration of thickener in the emulsions the stronger was the perception. Oral coatings perception was further influenced by the protein used in the emulsions, with Na-caseinate stabilised emulsions creating coatings with higher perception on creaminess and fatty-film.

Chapter 2 and chapter 3 provided knowledge on the deposition and clearance of oil coatings, but little was known on the formation of oil coatings. Chapter 4 focused on the formation of oil coatings formed by Na-caseinate stabilised o/w emulsions (1-20% (w/w)). The formation of oil coatings was a rapid process, where the maximum oil deposition was achieved at normal drinking behaviour (~3s). Further, in Chapter 4 we investigated the hypothesis often referred on literature, in which oil coatings form a physical barrier which prevents tastants to

reach the taste buds, and thus create a reduction on taste perception. It was concluded that oil coatings formed by emulsions within one sip did not affect subsequent sweetness perception of sucrose solutions. We suggested that the oil droplets deposited on the tongue (as seen on chapter 2) did not form a hydrophobic barrier that is sufficient to reduce the accessibility of sucrose to the taste buds and consequently does not suppress taste perception.

Previous chapters focused on oral coatings formed by liquid o/w emulsions, however studies describing oral coatings formed by semi-solids and solids are scarce. As such, chapter 5 focused on the formation, clearance and sensory perception of fat coatings from emulsion-filled gels. Four emulsion-filled gelatin gels varying in fat content and type of emulsifier (whey protein isolate - created fat droplets bound to matrix; tween 20 - created fat droplets unbound to matrix) were studied. As in for oil coatings formed by liquid o/w emulsions, fat coatings formed by emulsion-filled gels reach their maximum deposition in the first seconds of mastication. This suggests that the first bites are the most relevant for the formation of fat coatings on the tongue. Further, fat fraction deposited on tongue increased when oral processing time of the gels increased. This trend was clearer for gels with higher fat content (15%) compared to gels with lower fat content (5%). Fatty perception increased with increasing mastication time, and decreased after expectoration with increasing clearance time. Fat fraction deposited on tongue and fatty perception are higher in gels with unbound droplets compared to bound droplets, as well as in gels with 15% fat compared to 5% fat.

To elucidate the role of protein on oral coatings, Chapter 6 focused on the development of a method to quantify protein in the oral coatings. Further, Chapter 6 studied the influence of protein content, in-mouth protein behaviour (lysozyme - protein which creates flocs with saliva vs. Na-Caseinate - protein which does not create flocs with saliva) and presence of thickener on the formation of protein oral coatings and sensory perception of protein coatings. Protein coatings were collected from the front and middle part of the anterior

tongue using cotton swabs after subjects orally processed protein solutions for different time periods. Protein concentration of the coating (mass protein/mass coating) was quantified with the Lowry method. Similarly to oil/fat coatings, results show protein coatings are formed rapidly, reaching maximum deposition on the first seconds of the samples´ oral processing. Further, different protein in mouth-behaviour (Na-caseinate *vs.* lysozyme) did not create differences on protein deposition on the tongue. Presence of xanthan-gum in the processed samples decreased protein deposition on the tongue, compared to when samples without xanthan-gum were processed. The perception of protein coatings was strongly influenced by the viscosity and protein used in the samples. Higher viscosity of the samples lead to higher intensity on creaminess and thickness. Lysozyme samples created coatings with high sweetness and astringent intensity, which is related to the molecular structure of the protein.

Changes in the viscosity of beverages can cause changes in thickness perception. The changes in thickness perception can be accompanied by differences in other sensory properties, such as sweetness and creaminess which might be undesirable when reformulating beverages or developing new products. Knowledge on the differences by which viscosity of beverages can be modified to create a difference in sensory perception is currently lacking. Chapter 7 focus on the determination of the Just Noticeable Difference (the minimal difference that can be detected between two stimuli) for thickness perception of beverages. Oral thickness sensitivity (K=0.26) was found to be comparable to literature values for kinesthetic food firmness and spreadability, creaminess, sourness and bitterness perception.

The aim of this thesis was to determine and characterize factors influencing oral coatings and their sensory perception. For this purpose, reliable methods to quantify oil and protein deposited on the tongue had to be developed to later study the macronutrients deposition. Further, the influence of stimulus properties on the formation and clearance dynamics of oral coatings and their impact on sensory perception were investigated.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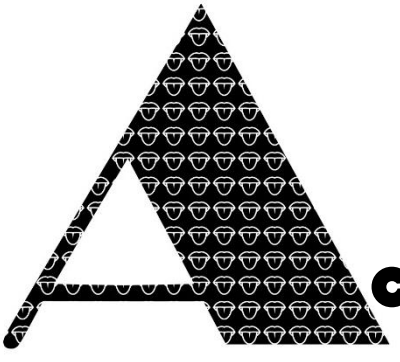

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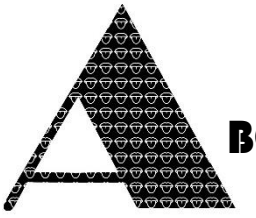


BOUT THE AUTHOR

Sara Camacho was born in Madeira, Portugal on 3rd December, 1985. She grew up in Funchal until finishing her high school studies. Afterwards, Sara moved to Lisbon to study at Instituto Superior Técnico, Universidade Técnica de Lisboa. She graduated with an Integrated Master of Sciences in Biological Engineering. She visited The Netherlands for the first time for her MSc thesis on "Taste enhancement by pulsatile delivery of food-models". Sara´s thesis had a duration of 6 months and her work was carried out at NIZO food research, Ede in 2009. In 2010 she returned to NIZO to conduct experiments on taste and aroma perception under the supervision of Dr. Kerstin Burseg.

In 2011 she was appointed as a PhD student within the project: "Dynamics of Texture Taste Perception" funded by Top Institute Food and Nutrition. The PhD project was conducted in the Sensory Science and Eating Behaviour Group and the Food Quality and Design Department at Wageningen University under supervision of Dr. Markus Stieger, Dr. Fred van de Velde and Prof. Dr. Kees de Graaf.

LIST OF PUBLICATIONS

Formation dynamics of oral oil coatings and its effect on subsequent sweetness perception of liquid stimuli. **Camacho S**. van Eck A. van de Velde F. Stieger M. Published J. Agric. Food Chem., 2015, 63 (36), pp 8025-8030

Formation, clearance and mouthfeel perception of oral coatings formed by emulsion-filled gels. **Camacho S** *, Liu K *, van der Linden A, Stieger M, van de Velde F. Accepted J. Food Texture 2015

Just Noticeable Differences and Weber Fraction of oral thickness perception of model beverages. **Camacho S**, Dop M, de Graaf C, Stieger M. J. Food Science (Sensory and Food Quality), 2015, 80 (7), pp 1583-1588.

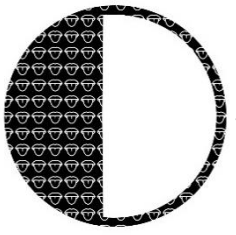
Properties of oil/water emulsions affecting the deposition, clearance and after-feel Sensory perception of oral coatings. **Camacho S**, den Hollander E, van de Velde F, Stieger M. J. Agric. Food Chem . 2015, 63 (8), pp 2145–2153

Physical and sensory characterizations of oral coatings of oil/water emulsions. **Camacho S**, van Riel V, de Graaf C, van de Velde F, Stieger M. J. Agric. Food Chem. 2014, 62, pp 5789-5795.

Taste enhancement by pulsatile stimulation is receptor based but independent of receptor type. Burseg KMM, **Camacho S**, Bult JHF. Chemosens Percept 2012 Jun 27;5(2), pp 179-187

Effects of pulsation rate and viscosity on pulsation-induced taste enhancement: New insights into texture-taste interactions. Burseg KMM, **Camacho S**, Bult JHF. J. Agric. Food Chem. 2011, 59, pp 5548–5553.

Sweet taste intensity is enhanced by temporal fluctuation of aroma and taste, and depends on phase shift. Burseg KMM, **Camacho S**, Knoop J, Bult JHF. Physiology and Behaviour 101(2010) pp 726-730.



VERVIEW OF COMPLETED TRAINING ACTIVITIES

Courses

Food structure and Rheology, Wageningen University, 2012

Advanced food Analysis, Wageningen University, 2013

Industrial Food Proteins, Wageningen University, 2013

Nutriscience, Wageningen University, 2013

Regulation of energy intake: the role of product properties, Wageningen University, 2013

Conferences

7th NIZO Dairy conference, The Netherlands, 2011 (poster presentation)

2nd Food Oral Processing Conference, France, 2011

10th Pangborn Sensory Science Symposium, Brazil, 2013 (poster presentation)

Food Structure & Functionality Forum Symposium, The Netherlands, 2014 (oral presentation)

3rd Food Oral Processing Conference, The Netherlands, 2014 (oral and poster presentation)

Delivery of Functionality in Complex Food Systems, France, 2015 (oral presentation)

General Courses

VLAG PhD week, 2012

Scientific Writing, Wageningen University, 2013

Sensory statistics course, Nottingham University, 2012

Techniques for Presenting and writing a paper, Wageningen University, 2014

Career Perspectives, Wageningen University, 2014

Voice Matters - Voice and Presentation Skills Training, Wageningen University, 2015

Optional Courses and Activities

Preparation PhD research proposal

PhD Trip, Singapore and Thailand, 2014

Colophon

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