The Fate of Fat: Tribology, Adhesion and Fat Perception of Food Emulsions

Promotor:

Prof. Dr. M.A. Cohen Stuart

Hooglereaar Fysische chemie met bijzondere aandacht voor kolloïdchemie

Wageningen Universiteit

Copromotoren:

Dr. E.H.A de Hoog

Wetenschappelijk onderzoeker/ projectleider

NIZO Food Research, Ede

Dr. G.A. van Aken

Senior wetenschappelijk onderzoeker/ projectleider

NIZO Food Research, Ede

Promotiecommissie:

Prof. Dr. Ir. M.A.J.S. van Boekel	Wageningen Universiteit, Nederland
Prof. N.D. Spencer	ETHZ, Zürich, Zwitserland
Dr. R.H. Tromp	NIZO Food Research, Ede, Nederland
Dr. L.N. Arnaudov	Unilever Research, Vlaardingen, Nederland

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The Fate of Fat: Tribology, Adhesion and Fat Perception of Food Emulsions

Diane M. Dresselhuis

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General introduction

Obesity is an increasing problem in the modern Western Society. Therefore, the food industry has worked for years on developing food systems that have reduced energy densities while still being highly appreciated for their flavour. Whereas reducing carbohydrate levels by using non-caloric artificial sweeteners and non-starch thickeners has been largely successful, reducing fat levels without loosing the products' fatty, creamy sensation has been very difficult.

Several products have been launched that are labelled "low fat". These products contain, for instance, fat substitutes such as the sucrose ester 'Olestra', the whey protein based product 'Simplesse' or the recently launched whey protein based product 'DOMO Hiprotal 60MP'. Another way to reduce fat is to replace part of the fat by a thickening agent. However, replacing fat by one of these ingredients or adding a thickener does not compensate entirely the loss in fatty/creamy sensation, which occurs as a result of reducing fat levels.

A more systematic approach in developing low fat food products is needed. The questions to be addressed are (i) how is fat sensorially perceived by the consumer and (ii) how can food ingredients and the texture of the food influence this perception. Since the perception of food largely takes place in the mouth, studying the influence of oral processing on product properties is an essential step. For these kind of studies one preferably selects products, which are (i) traditionally high in fat and (ii) consumed regularly, so that the impact of reducing fat levels on a person's total fat intake is significant. Among food products, food emulsions meet these requirements. By investigating the effect of oral processing on both product characteristics as well as on perception we hopefully can obtain valuable information on how to engineer low-fat food emulsions.

1.1 Oral surfaces and saliva

'Oral processing' is the term used here to describe the complete process from ingestion, mastication until swallowing of food. Mastication can be defined as the process by which food taken into the mouth is converted into a form suitable for swallowing [1]. The research in this thesis is limited to oil-in-water emulsions of which the continuous phase is not thickened or gelled by thickening agents such as starch, carrageenan or xanthan. Low viscosity food emulsions are already in a suitable form for swallowing. Yet, liquid emulsions still undergo a mastication phase before they are swallowed. It is believed that this occurs to check the palatability (e.g., suspected poisonousness or bacterial contamination), to change the temperature to body temperature, and to mix the emulsion with saliva for, e.g., buffering reasons [1]. When a person is asked to judge an emulsion sensorially, the mastication time tends to become longer. In that case extra smearing of the emulsion over the palate is observed [2]. Prior to the last stage of oral processing, swallowing, the emulsion is pressed by the tongue against the palate and is squeezed towards the oesophagus. In general, swallowing of liquid systems occurs within 2-3 seconds after ingestion [3]. Hence, this is the time window in which (fat) perception takes place.

Contact of the emulsion with both saliva and oral surfaces is very important for food perception. Especially the tongue plays a major role in food handling and perception. The human tongue consists of various papillae, which differ in size and function and which are distributed inhomogeneously over the tongue surface (see Figure 1.1). Although the human tongue has been studied in connection to various infections, inflammations, cancers, etc., fundamental understanding of the normal structure and functionality of the healthy tongue is limited [4]. A function that has been widely studied is the detection of the primary taste sensations: sweet, sour, bitter, salty and umami. Humans detect these sensations through interaction between taste molecules and receptors present at the cells of the taste buds, which are localized in the papillae [5]. Among the various papillae, the filiform papillae are special since they do not contain taste buds, cover almost the whole surface of the tongue and give the tongue its roughness, thus allowing food handling. The filiform papillae are cone-shaped and have a keratinised tip [4]. They are expected to contain mechano-receptors, which are thought to play a role in sensing the texture of food.

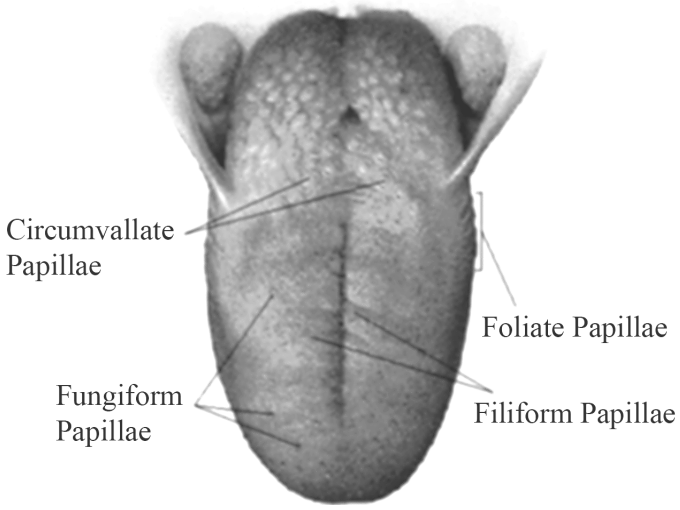

Figure 1.1: Distribution of papillae over the tongue surface (image derived from [6])

Mechanoreceptors respond to tactile stimuli, such as pressure, and are mainly found in the human skin [7]. Information on mechanoreceptors in the tongue is scarse. However, there are some studies which have identified superficial and deep tongue receptors [8; 9]; recently also the location where the signals are processed in the brain [10] has been studied. Although mechanoreceptors in the tongue are not fully understood a relation with food texture perception has been proposed [11-13]. Instead of 'oral tissue' also often the term 'oral mucosa' is used. 'Mucosa' refers to the type of epithelial tissue, which is found along, e.g., the whole intestinal tract (including the mouth), the reproductive, and the respiration tract.

Saliva is a highly complex aqueous fluid containing proteins (0.3%) and trace substances (0.2%) such as electrolytes [14]. Over 300 different proteins have been identified, mainly glycoproteins, enzymes, immunoglobulines and a wide range of peptides [14]. Mucins make up a large percentage of the protein content in saliva. They form a specific group of proteins that are disulphide-linked and contain heavily glycosylated domains, alternated with less glycosylated ones, as well as sialic acid side chains [15]. Mucins are very surface active, they can emulsify oil [16].

Two secreted mucins are found in human saliva, MUC5B (formerly named MG 1) and MUC7 (also named MG 2) [15]. Both are high molecular weight proteins with molar masses of 41000 kDa and 200-300 kDa, respectively [17; 18]. These mucins are mainly

secreted by the submandibular and sublingual salivary glands located under the tongue. It has been suggested that also membrane-bound mucins (MUC4) exist in the mouth [19].

The secreted mucins are found to interact with emulsions in the bulk, causing flocculation. Depending on the pH, the proposed mechanisms are electrostatic and depletion interaction [15; 20]. In the mouth, emulsions can also interact with mucins which cover the oral surfaces. In the body, mucins are present in tear fluid and in the intestinal, reproductive and respiration tract. Since the functionality of the mucus layer is different in each part of the body, also the characteristics of this layer will vary. It has been widely reported that on the epithelial cells of these body parts mucins form a ”gel-like” layer. However, it is not clear whether such a gel-like mucus layer is also present at the tongue surface, or whether mucins adhere to the surface only forming a thin protein layer with a thickness of several nanometers. Furthermore, besides the binding of MUC4, which has been suggested to be covalently bound, hardly any information is available on the physical origin of the interaction between mucins and epithelial surfaces and the strength of the interaction [19]. Therefore, in the context of this thesis we initially consider two situations: (i) emulsion droplets interacting with the tongue surface through interaction with mucins (or other salivary proteins) adhered to the surface in a thin layer (interaction like in the bulk [15; 20]) and (ii) emulsion droplets interacting with the uncovered, (bare) epithelial cells of the tongue surface.

1.2 Sensory perception

When a food emulsion is or has been in contact with the oral surface it can be perceived as, e.g., ‘fatty’ or ‘creamy’. An important question is how is ‘fat’ detected in the mouth. Other than for the basic tastes sweet, sour, salt, bitter and umami, there is no conclusive evidence on the presence of a receptor dedicated to perception of fat at the human tongue. In studies performed with rodents a CD36 receptor sensitive to fatty acids has been found [21; 22], which is expected to be involved in fat perception. However, this receptor has not been proven to be present on the human tongue.

Receptor or not, humans are apparently perfectly capable of detecting the ‘flavour’ of fat. When we refer to ‘flavour’ we use, within the context of this thesis, the definition proposed by de Roos [23], who defines flavour as the result of the combined effect of

aroma (or odour), taste and mouth-feel. 'Aroma' is related to perception of volatiles present in the vapour phase above a food product, which humans can detect orthonasal or retronasal. 'Taste' refers to the basis taste stimuli discussed earlier (sweet, sour, bitter, salt and umami). Finally, mouth-feel results from tactile sensations, which can be perceived by touch, and allow perception of differences in texture, structure and temperature. As discussed earlier, the filiform papillae are suggested to play a role in tactile sensations.

Fat perception is the result of a complex interplay between mouth-feel and aroma sensations [24; 25], but also individual preferences, demography [24] and memory [26] are proposed to play a role. In this thesis the main focus lies on mouth-feel related to fat perception.

For years the main focus of texture (mouth-feel) related research was on the rheological behaviour of food and its effect on (fat) perception. Perception of fat (or creaminess) was found to be related to the apparent viscosity [27-29]. However, recently various authors suggested that rheological behaviour alone cannot predict fatty (or creamy) mouth-feel of emulsions, and that the ability of emulsions to reduce in-mouth friction is an important aspect. They suggested that there is a relation between orally perceived friction and sensory fat perception [30-34]. During and after consumption of a food emulsion, humans tend to rub their tongue over their palate, which generates friction forces. Considering in-mouth friction as an important factor in fat perception is a promising new approach to understand creamy and fatty sensations. However, only recently research groups [33-35] started to perform systematic research on this subject.

1.3 Oral Tribology

Tribology is the science that studies friction, lubrication and wear phenomena. It is mainly studied in connection to high pressure processing of, e.g., metal. Friction is the force between interacting surfaces that resists and hinders their relative movement. A differentiation is made between static and dynamic friction. Dynamic friction is the mechanical force between sliding and rolling surfaces that resist the movement. Static friction must be overcome to start the relative movement between two bodies [36]. The magnitude of the friction force (F_f) is determined by the true contact area and the applied normal load (F_N). Friction is generally expressed as a friction coefficient μ (eq 1.1).

$$\mu = \frac{F_f}{F_N} \qquad (1.1)$$

The contact area, and thus the friction force, depends on numerous material parameters such as the roughness, the deformability and the visco-elasticity of the surfaces. Friction also depends on, e.g., adhesion phenomena between the two sliding surfaces, the presence of a lubricant, the characteristics of this lubricant, the interaction of the lubricant with the surfaces and the speed of shearing. Due to the dependency of friction on so many factors, which also influence one another, predicting μ quantitatively is almost impossible. Most often an experimental curve, a so-called Stribeck curve (Figure 1.2), is measured to characterise the behaviour.

The Stribeck curve is determined by shearing two surfaces in relative motion over one another at various speeds while simultaneously measuring the friction force. Often a lubricant is present to reduce the friction.

The Stribeck curve has a characteristic shape, which reveals three regimes. In the 'boundary regime' the separation between the surfaces is smaller than the asperities of the surfaces. Here, μ is hardly affected by the sliding speed but is mainly determined by the chemical constitution of the thin lubricant films covering the solid surfaces [36]. These films can be a few molecules thick.

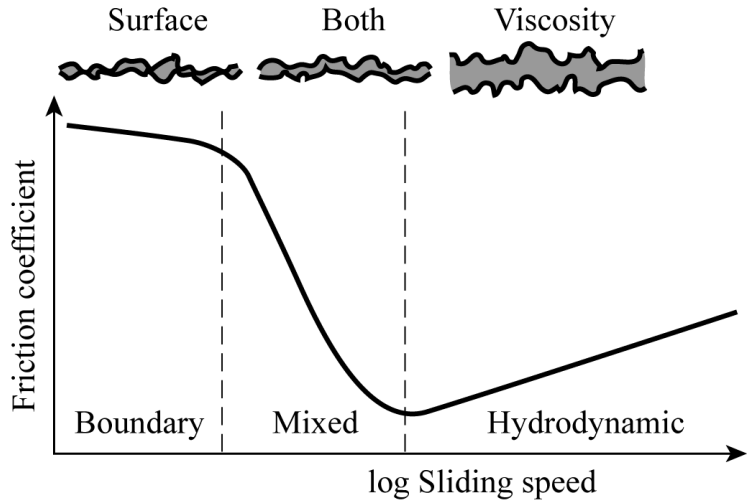

Figure 1.2 Stribeck curve with the three lubrication regimes boundary, mixed and hydrodynamic lubrication.

The 'hydrodynamic regime' has been most extensively studied. In this regime the surfaces are completely separated due to the build up of hydrodynamic pressure. This

results in a friction force typically 100 times smaller than in the boundary regime [36]. The friction force in this regime depends on the relative speed (v), and on the viscosity (η) of the lubricant, and can be calculated using the Navier-Stokes equation (1.2) provided the flow of the lubricant is laminar and the fluid is Newtonian [36]:

$$F_f = \frac{A}{d}.\eta.v \quad (1.2)$$

Here, A is the surface area of two parallel plates, and d the thickness of the lubricating layer. This equation shows that by decreasing the viscosity of the lubricant one can lower the friction. However, the real situation is more complicated since the viscosity should be high enough to maintain a lubrication film with a thickness exceeding the height of the asperities, thereby avoiding the solid surfaces to make contact [36]. Hence, in practice, lowering the friction by lowering the viscosity is limited.

In between the boundary and the hydrodynamic regime there is a crossover area called the 'mixed regime', in which intermittent contact between the surfaces occurs. The boundary and mixed regimes have not received so much attention, since these are the regimes of relatively high friction and in the context of, e.g., high-pressure metal processing these regimes are preferably avoided (high friction at high-pressure means high energy consumption and a large amount of wear). This lack of attention for the boundary and/or mixed regime is also the reason why conventional tribometers are often especially designed to use smooth metal surfaces and to reach high speeds, making circular movements. Furthermore, often high normal loads are applied. To study in-mouth friction these tribometers are less suitable. First of all, the tongue may be expected to operate in the boundary and/or mixed regime since food handling by the tongue requires sufficiently high friction between the tongue and the ingested food. This is presumably also the reason why the tongue is rough rather than smooth. Furthermore, the tongue makes a sliding, non-circular, movement at considerably lower speeds and pressures than those used in, e.g., metal processing or engines, and the tongue has a much lower Young's modulus than metal. Because of these differences between, e.g., metal processing and consuming an emulsion, we need a tribometer, which is adapted to measure under in-mouth conditions, enabling determination of in-mouth friction.

1.4 Adhesion on surfaces

The tongue is expected to be boundary and/or mixed lubricated when non-solid food is consumed. This implies that the adherence of lubricant components at the tongue surfaces determines to a large extent the friction and therefore presumably the sensory perception. These adhered components can form a layer that can either increase or decrease friction. A decrease in friction occurs when adhesive forces between the lubricant layers present at the solid surface are lower than those between the bare solid surfaces [36]. In the opposite case the friction (e.g. surfaces covered with a glue) increases.

Depending on the solid surface characteristics, emulsion components can adhere to the surfaces and lower the friction. Despite the fact that oil-in-water emulsions are frequently used in lubrication of solid surfaces (e.g. metal surfaces), the mechanism how surfactant-stabilised emulsions lubricate (metal) surfaces is not well understood, and therefore the design of lubricating emulsions is still very empirical [37]. Studying lubrication of the complex oral surfaces by protein-stabilised food emulsion and the role of adhesion has, to the best of our knowledge, not been studied before.

1.5 Stability of food emulsions

The stability of emulsion droplets under oral processing is expected to influence lubrication of the oral surfaces. The term ‘stability’ refers to both physical and chemical phenomena. Physical instability of emulsion droplets (e.g. coalescence, flocculation, creaming) results in alteration in the *spatial distribution* of molecules, whereas chemical instability (e.g. hydrolysis, oxidation) results in alteration of the *kind* of molecules [38]. In practice, it is hard to distinguish between the different aspects of instability. Since swallowing takes only a few seconds, we expect that mainly physical instability such as (shear-induced) coalescence is important for lubrication of the oral surface. However, in some cases also chemical instability due to enzymatic degradation can play a role in emulsion lubrication. The question now is: what determines the physical instability (sensitivity towards coalescence) of protein-stabilised food emulsion droplets in the mouth?

Coalescence of emulsion droplets is determined by two main aspects. The first aspect is the opportunity for droplets to come close together for a definite time. Therefore, the interaction potential between droplets and the frequency of collisions is of importance. Aggregation of emulsion droplets or close packing of droplets within, e.g., a sedimentation layer, often precedes coalescence. However, there are also emulsions of which the droplets at close separation distance remain stable against coalescence for years [39; 40]. This is determined by a second factor, namely the resistance of the interfacial layer against rupture or spontaneous hole formation [41]. The exact mechanisms underlying film rupture are still poorly understood [42] and despite the large amount of fundamental research in this area, still under discussion (see [43] and references therein). A number of potential causes for film rupture have been reported by van Aken [43]; they include rupture due to insufficient adsorption of surfactant at the droplet oil/water interface, rupture by spontaneous oil neck formation between droplets, and rupture by film stretching. Especially the latter is suggested to play a role in coalescence of protein-stabilised emulsions; its occurrence depends on the characteristics of the adsorbed layer as well as on the externally applied forces, such as hydrodynamic forces due to centrifugation or pumping during manufacturing [41; 43].

Proteins are reported to form interfacial layers, which provide (i) good mechanical stability against rupture and (ii) a barrier in the disjoining pressure (π) which opposes droplets approaching one another [42; 43]. This disjoining pressure is at long separation distances (> 10nm) the sum of the Van der Waals and electrostatic interaction forces. At short separation distances (< 10nm) also steric and hydrophobic interaction forces can play a role [38].

In this thesis whey protein isolate (WPI) is used to stabilise sunflower oil droplets, since these are ingredients often used in food products. Whey protein isolate contains a mixture of globular proteins (β-lactoglobuline ~55%, α-lactalbumine ~24%, serum albumin ~5% and immunoglobuline [44]). Whey proteins are capable of forming an adsorbed interfacial protein layer with a thickness of several nanometers, which provides steric repulsion and resistence against rupture [45]. Whey proteins also provide electrostatic repulsion between droplets. At the pH of most food emulsions (~ neutral pH) the emulsion droplets stabilised by whey proteins are negatively charged (IEP ~4.5 [46]).

Coalescence of emulsion droplets can be induced by various factors. In this thesis, coalescence of protein-stabilised emulsions in the mouth and the possible relation with lubrication of the tongue surface is investigated. Aspects of oral processing that are expected to influence the occurrence of in-mouth coalescence are: shear flow, presence of saliva causing flocculation [20], incorporation of air [47] and contact with the oral surfaces. The effect of these processes on actual in-mouth coalescence, also as function of emulsion characteristics, constitutes the theme of this thesis work.

1.6 Aim and outline of this thesis

The aim of this thesis is to unravel how oil-in-water food emulsions interact with (oral) surfaces and what the influence of this interaction is on fat perception. As may be concluded from the discussion above this interesting subject needs an interdisciplinary approach. It is fair to say that at the start of this thesis not only I was new to the research field, but also the whole research field was new and just starting up. Therefore, we first needed to set up methods, define the system and verify assumed dependencies.

To be able to verify the hypothetical dependency between fat perception and in-mouth friction, a mouth-mimicking tribometer (Figure 1.3), the Optical Tribological Configuration (OTC) was constructed. This set-up allowed to shear lubricants (A) such as emulsions between a mouth-mimicking surface (B) and a glass plate (C) while simultaneously observing emulsion behaviour with a Confocal Scanning Laser Microscope and measuring friction forces.

The OTC is mouth-mimicking in the sense that the lubricant is sheared between the upper plate (B) and lower plate (C) in a parallel oscillatory movement, similar to the tongue rubbing over the palate. As suggested earlier, this is essentially different from commercial tribometers, which make a circular instead of a parallel movement. Moreover, in case of commercial ball-on-disk set-ups the ball rolls rather than slides over the surface, which is totally different from any in-mouth situation. Furthermore, the normal forces (F_N) applied in the OTC are lower than those in commercial tribometers (0.5 N and lower, vs. 3 N and higher in commercial set-ups) and comparable with the tongue pressing against the palate. By pressing the tongue with maximum force against a force transducer it was estimated that humans can approximately generate a tongue pressure of 60 kPa,

which is of the same order of magnitude as what one gets by applying 0.5 N with the spherical shaped upper plate (B) of the OTC on the lower plate (C). Finally, the OTC allows to impose mouth-relevant speeds and, very important, to use mouth-relevant surfaces such as pig's tongue in addition to modified artificial surfaces (which can be used as upper plate (B)).

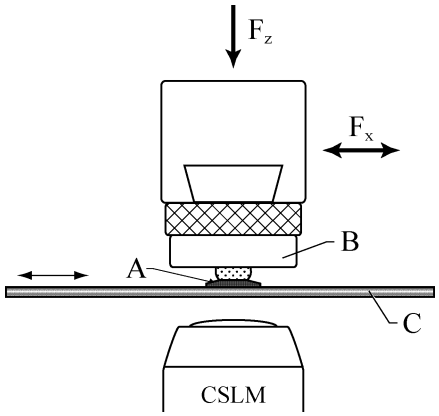

Figure 1.3: Optical tribological configuration (OTC); an emulsion (A) is confined between an upper changeable surface (B) and a glass surface (C). A force (Fz) is applied and the friction force (Fx) is measured, while surface C is oscillating. Simultaneously with a Confocal Scanning Laser Microscope (CSLM) the behaviour of the emulsion is observed.

In order to carry out this thesis work, I needed to find a surface representative for the mouth, which was available in large quantities (to allow for numerous experiments) and which could be attached to the force transducer of the OTC. **Chapter 2** compares a pig's tongue and a PDMS surface for their capability to mimic the human tongue. Both surfaces are characterised in terms of surface hydrophobicity, roughness and deformability. The influence of the differences in these characteristics on emulsion behaviour and friction is studied using the OTC. Also, preservation methods for pig's tongue are investigated to minimize retardation processes (which could lead to false conclusions on in-mouth emulsion behaviour) as well as measuring methods for the OTC.

Observations in Chapter 2 and in previous work [48] suggested that in-mouth coalescence of emulsion droplets might influence in-mouth friction. This is further investigated in **Chapter 3**, in which emulsions with an expected variation in sensitivity towards coalescence were sensorially evaluated on fat perception and orally perceived friction. These sensory data were combined with *ex vivo* friction measurements performed with the OTC (using pig's tongue). Also, the actual sensitivity towards (in-mouth) coalescence was determined using various techniques.

The mechanism by which emulsions can lubricate the oral surface was studied in more detail in **Chapter 4** by determining the influence of both emulsion characteristics and surface characteristics on the friction, using the OTC. Modified polydimethylsiloxan (PDMS) served as a mouth-mimicking solid surface. It was modified in terms of roughness, hydrophobicity and deformability. The various lubricants included emulsions varying in sensitivity towards coalescence, pure oil and water.

It is expected that the *ex vivo* determined ability of various emulsions to lower the friction (Chapter 4) also results in an *in vivo* difference in fat retention at the tongue (after expectorating the emulsion). This was investigated in **Chapter 5** and included also *ex vivo* experiments using pig's tongue, which were performed to study the influence of saliva on fat retention. In this chapter several fat detection techniques are applied which are adapted to enable the measurement of oral fat retention. Also, the possible mechanisms, as proposed in Chapter 3 and 4, namely that emulsions lubricate the tongue through adherence and spreading, are discussed by considering the colloidal forces that could be involved. The obtained fat retention data were discussed in terms of these colloidal forces.

Finally, **Chapter 6** is a direct follow-up study of Chapter 5 and is focussed on applying a method that proves that indeed droplet adhesion and spreading do occur when electrostatic, steric and hydrophobic interactions between droplet and solid surface are favourable. So far, studies on adhesion of stabilised emulsion droplet on solid surfaces were rare. Spreading of protein-stabilised emulsion droplets on solid surface was to our knowledge not even reported before. In this chapter the implications of all these findings, and the findings of previous chapters are rationalized in terms of colloidal forces, friction and fat perception.

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2

Application of oral tissue in tribological measurements in an emulsion perception context

Abstract

Tribological measurements are indicated to be a tool in predicting the creamy in-mouth sensation of a food product. Tribological measurements relating lubricational behaviour of a food product to perception are often conducted with artificial surfaces. In this work we used pig's tongue to mimic the human tongue, which has the advantage of having surface characteristics similar to a human tongue. Using biological material also has some drawbacks. The most important drawbacks are the limited availability, the individual differences between the tongues, and the relative fast degradation of the tissue. The aim of this study was to identify the characteristics of the tongue in terms of surface roughness, deformability and wetting properties. The knowledge on these characteristics can serve as reference when using modified PDMS in tribological experiments relating perception to in-mouth friction. Furthermore, we demonstrated that knowing these characteristics is crucial for drawing rightful conclusions in tribological studies. Tribological measurements were performed with an experimental set-up combining friction measurements with CSLM observations. We identified the importance of these characteristics for tribology measurements performed in relation to sensory perception.

It is shown that the tongue surface has some very typical characteristics, including the presence of papillae, a hydrophilic mucus layer, and an elastic modulus that is at least two orders of magnitude smaller than that of smooth PDMS surfaces. The different surface characteristics appear to lead to completely different lubricational behaviour of the food emulsions between these surfaces. Furthermore, for food emulsions differences in the occurrence of coalescence were found between shearing with pig's tongue and PDMS surfaces. Therefore, we conclude that for studies relating sensory properties of food systems to lubricational behaviour, a careful choice of representative surfaces is essential and that modification of smooth PDMS can result in surfaces having characteristics better resembling tongue tissue characteristics.

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2.1 Introduction

Reducing the fat content of food products without a considerable loss of the desired creamy taste has been a great challenge for both industry and food research. To improve the understanding in this, many studies have attempted to relate fat-related sensory attributes, such as creaminess and fattiness, to rheological and chemical properties of food systems.

Creaminess appears to be a highly integrated and complex perception, of which the relation with emulsion characteristics is disputed. Various studies indicate that the creaminess of an emulsion is related to its apparent viscosity [1-3], whereas other studies did not reveal such a relationship [4]. At the level of brain activity, Verhagen and co-workers monitored neuron signals in the brain of a rhesus macaque. They assessed that viscosity and fat can be perceived independently [5], which indicates that creaminess is not only related to viscosity. Others suggest that not only mouth feel attributes such as viscosity, but also aroma is of importance for the creamy sensation [4; 6-8].

The fact that sensory properties of emulsions are not simply related to viscosity is, at least partially, due to the complexity of oral processing. Oral processing of a semi-solid food product affects the structure of the product by changing its temperature, mixing it with saliva, and exposing it to shear flow. Therefore, attempts to predict perception exclusively on the basis of rheological parameters measured outside the mouth are likely to fail. Moreover, an aspect that is not measured by rheology, but is probably sensorial relevant, is the process of rubbing and squeezing the product between tongue and palate during which the tongue and palate surfaces are partial in contact. In this process a friction force is generated between palate and tongue, with the semi-solid food acting as a lubricant. The oral friction can be described with the boundary or mixed friction regime, which implies that the surfaces are respectively in contact or partially in contact with one another. Friction has been reported by several authors to relate to the perception of creaminess [9; 10].

The lubricational behaviour of a food product is commonly analysed with standard tribology equipment such as a pin-on-disk or a ball-on-disk set-up. Using such equipment, one faces the problem of selecting a suitable material, representing oral tissue. In order to

mimic the oral mucosa, Lee and co-workers [11] used tetrafluorethylene (PTFE) and zirconia, respectively, as pin and disk. Malone and co-workers [9] used a steel ball and silicon rubber in their friction experiments. All these surfaces were smooth.

Although readily available, the drawback of using artificial surfaces such as rubber is that they have quite different characteristics from the oral mucosa. The tongue is not smooth, but is covered by various types of papillae of which the filiform papillae give the tongue its roughness. The filiform papillae are cone-shaped structures. Each of these papillae has a core of connective tissue covered by keratinised epithelium, which gives rigidity to the tissue. The interpapillary tissue is not keratinized [12]. Furthermore, the tongue consists of a complex interwoven 3D network of various skeletal muscles and fibre bundles, giving rise to complex deformation behaviour upon compression. The fibre bundles and skeletal muscles function together to produce a whole array of functional deformations either for speaking or for food handling [13]. Moreover, the tongue is covered by a mucus layer, consisting mainly of mucins (glycoproteins) and water. As a result, rubber is likely to differ from tongue tissue in physical characteristics such as surface morphology (e.g. various papillae), mechanical characteristics upon compression (simple elasticity vs. complex network of fibres), and wetting characteristics. In earlier work we demonstrated that the load dependence of the friction force is different for oral mucosa in comparison to artificial surfaces [14]. Several authors recognized that oral mucosa is quite different from artificial surfaces and modified their surfaces by tuning the roughness [15; 16] and the wetting characteristics [17] of the surface used in their experiments.

In this work we focus on the surface characterisation of tongue tissue and the importance of the surface properties on the behaviour of an emulsion as a lubricant. To this end, we studied three surface characteristics that are probably related to tribology in the mouth: surface morphology, deformability and wetting characteristics. We compared these surface characteristics of oral mucosa with the characteristics of PDMS, an artificial surface traditionally used in tribological measurements relating friction to perception. Although in reality the in-mouth lubrication behaviour of emulsions will also be influenced by the interaction with saliva [18], this aspect has not been included in the present study.

Tribological measurements were performed using a home-built tribological set-up, which measures the friction force between two surfaces in relative motion in horizontal direction. A Confocal Scanning Laser Microscope (CSLM) is connected to the tribological set-up, allowing microscopical observation of the behaviour of emulsion droplets sheared between various surfaces.

As a model surface for the human tongue, pig's tongue was used since both humans and pigs are omnivores, consuming roughly the same type of diet. It has been suggested that for studies investigating the structure of papillae, the oral mucosa of pigs is a better model system for human oral mucosa than that of rats [19]. However, because the oral epithelium of adult pigs is much more strongly keratinised than for humans [12] we used the less keratinised tongues of piglets of around 4 months old. Due to logistical reason and limitations in the availability of fresh tongue tissue the tongues were preserved. To use the tongue samples in the tribological experiments the effect of the storage conditions on the structure of tongue samples was studied.

2.2 Material and methods

2.2.1 Surface preparation and characterisation

Surface preparation

Tribological experiments were conducted with three surfaces: smooth glass, poly dimethyl siloxane (PDMS) and pig's tongue. PDMS (Sylgard 184 Dow Corning, USA) was prepared according to the supplier's procedure, mixing the PDMS base 10:1 with cross linker. To get PDMS with an even lower elastic modulus, the PDMS base was also mixed 20:1 with cross linker. The PDMS was cured in a 96-wells plate (Nunclon surface Nalge Nunc international, Denmark) to get spherical shapes with a diameter of 6 mm. The method to prepare PDMS surfaces was kindly provided by Seunghwan Lee and Nicolas Spencer of the ETHZ in Zürich (private communication). The glass cover slips (Chance Propper Ltd., West Mids, England) used in the tribology experiments had a roughness of ± 1 nm [20].

The pig's tongues were a kind gift by ID-DLO in Lelystad. The preparation and preservation of the tongues were optimized. Storing pig's tongues in appropriate media

just after sacrificing the pigs must minimize the altering of the tissue, which occurs as soon as the organism has died. Two solutions for storing tongues prior to preparation were tested: a physiological salt solution and a Tyrode solution. The Tyrode solution contains various salts, glucose, EGTA, Na-pyruvate and Hepes [21].

As is shown in Figure 2.1, only the anterior part of the tongue was used in the experiments. The tongue was cut in disc shaped samples with a diameter of 13 mm and a thickness of approximately 4 mm, before freezing the samples. The tongue samples were labelled A to J (Figure 2.1).

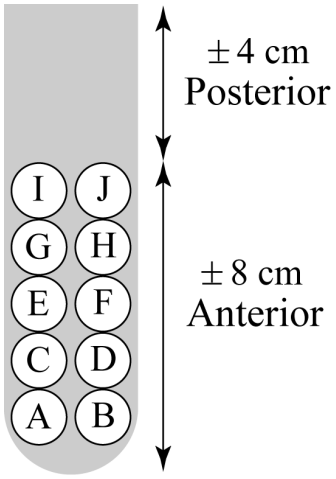

Figure 2.1: Schematic representation of a pig's tongue cut in disc-shaped samples taken from the anterior part of the tongue, labelled A-J.

To preserve the tissue, freezing media can be used to prevent ice crystal growth. Ice crystal growth causes, e.g., cell leakage. Since skin resembles mucosal tissue in terms of structure, skin preservation methods were tested to optimise the preservation. The methods described by Erdag et al, using freezing media containing glycerol or DMSO, were slightly altered [22]. The serum-free keratinocyte growth medium (KGM) was not added to the freezing media (in contrast to the procedure of Erdag et al) since the support of cell growth was not needed. In case of freezing samples in glycerol, the tongue samples were kept for 2 hours in a 15% glycerol solution at room temperature and then frozen in the glycerol solution. The samples frozen in a DMSO freezing medium were first placed in a 10% DMSO solution for 30 minutes and then for 10 minutes in 10% DMSO solution containing 0.5 M sucrose (incubating at room temperature) before freezing the samples in the DMSO solution containing sucrose.

Surface characterisation

The PDMS surfaces and the pig's tongues were characterised in terms of roughness, elastic modulus and wetting characteristics.

The roughness of pig's tongue was analysed using stereomicroscopy (Leica MZ16, Heerburg, Switzerland) and Confocal Scanning Laser Microscopy (CSLM). The CSLM set up used was a Perkin Elmer Ultraview RS confocal imaging system (Boston, USA), which was configured with a Scanning unit with a Nipkow disc, a CCD camera for detection, and an inverted microscope (LEICA DM IRBE, Heerburg, Switzerland) connected to an Ar/Kr laser (Melles Griot). The objective lens used was a 63x/UV/1.20 NA/water immersion/PL APO (Leica, Germany). To analyse the roughness of pig's tongues with CSLM, the tongues were stained with the fluorescent dye Rhodamine B. The excitation wavelength for this dye is 568 nm and the emission was detected between 580 and 620 nm. A 3D-image was constructed from multiple 2D-scans varying in depth. The roughness of the PDMS was determined with an atomic force microscope (AFM Dimension 3000, Digital Instruments, Santa Barbara, USA) using the contact mode. The mean asperity height determined with AFM, gives an indication of the surface roughness.

The elastic modulus of both PDMS and pig's tongue were determined by compressing the surfaces and monitoring the force in a Texture Analyzer (TA.XT plus, Stable Microsystems, Godalming, UK) and an Instron (Instron, Norwood USA). The spherically shaped tongue and PDMS samples were covered with paraffin oil to allow homogeneous compression and prevent barrelling. The compression speed was 5 mm/s. The Young's modulus was calculated from the stress-Hencky strain curve, using the first linear part of the curve over a range of 0-5% strain. Both PDMS and pig's tongue showed strain-hardening behaviour upon compression.

The wetting characteristics of the PDMS and tongue surfaces were determined by means of contact angle measurements. The automated drop tensiometer (ADT, ITConcept, Longessaigne, France) set-up was adapted to measure the contact angle of liquid on solid surfaces. To prevent evaporation of the water drop and drying out of the tongue surface, the PDMS and tongue surfaces were placed in a cuvet filled with sunflower oil. A 2µl water drop was automatically deposited at the surfaces using the ADT. The water contact angle was monitored in time using the camera of the ADT. The

contact angle was estimated by image analysis of the droplet shape.

2.2.2 Emulsions preparation and characterisation

A standard o/w emulsion containing 1 wt% Whey Protein Isolate (BiPro, Davisco Food International, USA; batch JE050-4-420) and 40 wt% sunflower oil (Reddy, Vandermoortele, Roosendaal, The Netherlands) was made. First a pre-emulsion was made, using an Ultraturrax (T25 basic, IKA, Staufen, Germany). Subsequently, the pre-emulsion was homogenised at 250 bar with a Panda Laboratory homogeniser (Panda NS1001L, Gea Niro Inc. Copenhagen) passing the homogeniser 10 times. The pH of the emulsion was 6.7. With light scattering (Mastersizer 2000, Malvern, Worchestershire, UK) the average particle size of the emulsion, *D[3,2]*, was determined to be 1.0 μm. A concentration of 0.02 (w/w)% sodium azide (Merck, analytical grade, Germany) was added to the emulsion to prevent bacterial growth. The viscosity of the emulsion was measured with a Physica MCR 300 (Anton Paar, Graz, Austria) using a cone-plate geometry with a diameter of 75 mm, a 1º angle and a gap of 50 μm. The emulsion showed shear thinning behaviour and had a viscosity of 6 mPa.s at a shear rate of 100 s^{-1}. Shear rates in the mouth are typically between 10-1000 s^{-1}, depending on the product [23]. No wall slip was observed when repeating the measurements. No aggregates or clusters were observed by light microscopy (Olympus BX60, Olympus Optical CO., Hamburg, Germany).

2.2.3 Friction measurements

Tribology measurements were performed with the Optical Tribological Configuration (OTC), see Figure 2.2. An amount of 150 μl emulsion was sheared in the OTC between two surfaces, PDMS/glass or tongue/glass, under a load (F_z) of 0.5 N. A load of 0.5 N was the maximum load, which could be applied to the tongue tissue without creating damage to the tissue. During each experiment the lower glass plate was oscillating 10 cycles over a distance of 16 mm, with a maximum speed of 80 mm/s. Simultaneously, the friction force (F_x) was measured and emulsion droplet behaviour under shear was observed with CSLM, making it necessary to shear against optical transparent glass cover slips. The average friction force was calculated over the range of

the movement where the speed of shearing was constant. Due to the limited availability of tongue tissue it was decided to limit the friction experiments to one load (0.5 N) and one speed (80 mm/s). Measuring at several speeds implies that more tongues would be needed since during the measurements the tissue alters due to wear and drying phenomena and needs replacement after every 2 speeds.

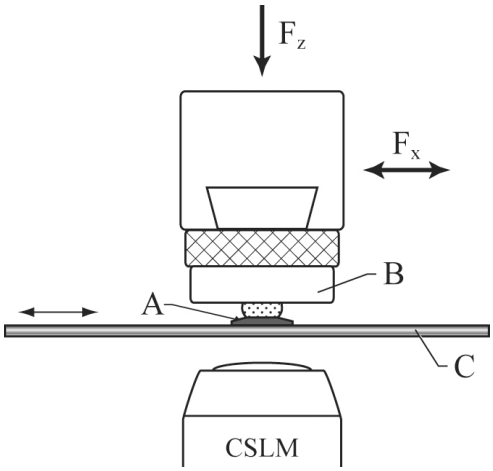

Figure 2.2: Optical Tribological Configuration (OTC); an emulsion (A) is confined between an upper surface, e.g. pig's tongue or PDMS (B) and a glass surface (C). A force (Fz) is applied and the friction force (Fx) is measured, while surface C is oscillating. With CSLM (§2.2.1) the behaviour of the emulsion is observed on a micrometer scale.

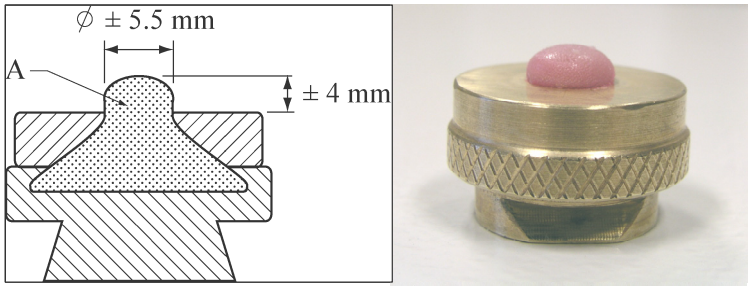

Figure 2.3: Schematic representation and picture of the OTC measuring probe with a tongue sample screwed into the probe.

The emulsion was stained with the fluorescent dye Rhodamine B, which has affinity for protein. CSLM images were taken at four stages: 1) before applying the load, 2) after applying a load of 0.5 N, 3) after shearing, still under compression 4) after the load had been removed.

Each experiment was carried out 5 times with tongue samples of different pigs, each sample taken at the same location on the tongue (see Figure 2.1). Shearing experiments with PDMS and glass, were carried out 3 times, using new PDMS samples for every experiment. Both the PDMS and tongue samples were screwed into the OTC

measuring probe in such a way that the samples were spherical-shaped (Figure 2.3).

2.3 Results and discussion

In this section we show the results of the characterisation of pig's tongue and we compare this with smooth silicone rubber (PDMS). We discuss the importance of these characteristics on the lubricational behaviour of an emulsion in tribology measurements. To be able to perform measurements with tongue tissue in a reproducible way, we first optimised the preparation and preservation of the tissue.

2.3.1 Pig's tongue preparation and preservation

Biological material is very susceptible to changes in structure after the organism has died. Both the method to prepare pig's tongues after sacrificing the pig, as well as the preservation method, must be optimised to prevent changes in the tissue structure influencing results obtained with the tissue in the tribological experiment. The aim of optimising the preparation and preservation of pig's tongue was not to perform an extensive physiological study on preservation methods, but to minimize, within the possibilities we have, the obvious retardation processes, which could lead to false conclusions on characteristics of tongue tissue. Ideally, experiments on fresh tongue samples would be preferred over preserved samples. However, this is very hard to achieve because of logistical limitations.

In biological and medical studies on the effect of changes in external conditions on tissue response, a Tyrode solution is often used to prevent cells from dying [21]. The effect of storage of tongues in a Tyrode solution, just after sacrificing the pig and before cutting the tongues, on visual appearance and elastic modulus was compared to the effect of storage in a physiological salt solution. Significant differences were not found; both solutions appear to be equally suitable for our purpose.

As indicated in Table 2.1, samples were stored without a medium and with two different freezing media, one containing glycerol and one DMSO. The samples were either directly placed in a -80°C freezer, or first put in liquid nitrogen to freeze them quickly (snap freezing) and then placed in a -80°C freezer. The same experiment was conducted, storing the samples at -20°C. The tongue samples had thicknesses of 8 mm.

Table 2.1: Tongue samples preserved without freezing medium, stored in a glycerol solution, stored in a DMSO solution. The samples are either placed directly in the -80º C or snap frozen in liquid nitrogen and then placed in -80º C. Young's modulus determined using a 1 mm/s compression speed, sample thickness was approximately 8 mm.

Preservation temperature -80 º C			Compression	
Sample	Medium	Method	Young's modulus (kPa)	St.dev. (%)
T1A	No	Direct	4.55	25.7
T1C	Glycerol	Direct	12.70	49.4
T1E	DMSO	Direct	9.82	32.7
T3A	No	Snap frozen	9.58	17.6
T3C	Glycerol	Snap frozen	7.42	25.3
T3E	DMSO	Snap frozen	3.99	30.3
T5A Fresh	No		15.30	28.6

After two and a half months the samples were analysed by performing compression tests with an Instron as well as observation of structural changes with stereomicroscopy. In general, the elastic modulus (Young's Modulus) was lowered by freezing and subsequent thawing of the samples, but the change was moderate, at most a factor of 3.4 (see Table 2.1). There is not a clear indication that one method is significantly better than the other. The stereomicroscopy images showed that freezing in glycerol or DMSO caused serious damage of the tissue since the epithelial layer became enlarged. This effect was more pronounced in the samples frozen at -20°C than in samples frozen at –80°C.

Based on the results of the preparation and preservation experiments it was decided to store the tongues in physiological salt solution immediately after sacrificing the piglets. The freezing media were supposed to prevent ice crystal growth, but unfortunately also altered the epithelial layer. By quickly freezing the samples in liquid nitrogen, ice crystal growth can be sufficiently delayed. Therefore, it was decided to preserve the tongues by first snap-freezing the samples in liquid nitrogen and subsequently storing them at -80°C without using a freezing medium.

2.3.2 Surface characteristics

Important characteristics of the surface, which are thought to influence the behaviour of the emulsion droplets, are the roughness of the surface, the deformability and the wetting characteristics.

Roughness

We distinguish macroscopic and microscopic surface roughness. The PDMS rubber used in our experiment had a microscopic roughness of 5 nm, as determined with AFM (data not shown). Tribological measurements described in literature relating perception to friction measurement do not report the roughness of the used rubbers [9-11; 24-26]; however, the roughnesses are likely to be similar to the PDMS surfaces used here. The mean asperity height of the elements on a tongue is much larger than on PDMS. The presence of mainly filiform papillae gives the tongue its macroscopic roughness. Figure 2.4 shows that the filiform papillae are approximately 320 μm long, the distance between them is about 300 μm and at the root of the papillae they have a typical thickness of roughly 120 μm.

Figure 2.4: Macroscopic roughness of pig's tongue: 3D CSLM image 300 x 500 μm (A), Stereomicroscopy image, scale bar is 500 μm (B), stereomicroscopy image, scale bar is 200 μm (C).

To evaluate the importance of the roughness of the tongue on emulsion droplet behaviour in the mouth, one should realise that food emulsions usually contain droplets with a size of at most a few micrometers, i.e., two orders of magnitude smaller than the macroscopic roughness of the tongue formed by the papillae. The radius of curvature of a papilla is large in comparison to the radius of curvature of an emulsion droplet. Therefore, at the length scale of the emulsion droplets the papillae are large bodies, which upon shearing will not be able to penetrate the interfacial layer stabilizing the droplets and to cause the droplets to rupture. However, the interaction of emulsion droplets with the microscopic roughness of the papillae surface could be of importance. Scanning electron microscope (SEM) pictures of human and pig's tongue show that, especially between the papillae, microplicae of the size of emulsion droplets are present [27]. When the radius of curvature of the microscopic asperities on the papillae is small in comparison to the radius

of curvature of the droplets, the contact pressure between droplet and asperity may become high enough to disrupt the interfacial layer. This may lead to penetration of the droplets by the surface asperities causing (shear-induced) coalescence and even fat release.

Moreover, we have indications based on CSLM-images that compression of the papillae creates confined spaces in which the droplets can become captured and concentrated, increasing their susceptibility to shear-induced coalescence. This ultimately may lead to oil release. So the possibility exists that, before shearing, the system consists of a water-dominated lubricant, containing particles (emulsion droplets), whereas after shearing a macroscopic oil phase can be formed, resulting in a lubricating mixture now consisting of water, emulsion droplets and oil. The change in the composition of the lubricating mixture can affect the friction force generated between tongue and palate. In order to judge how important the change in lubricating mixture is, we need to know whether the tongue surface is hydrophobic or hydrophilic (wetting) and how much oil is needed to change the friction between tongue and palate to the extent that it is perceived sensorially. More research using the OTC must be performed to verify this hypothesized behaviour of emulsions under shear.

Surface Deformability

The elastic modulus of asperities in the contact area is of importance in tribological measurements. In general, for small deformations the contact area is linearly proportional to the load and the elastic modulus [28]. In tribological measurements the friction force depends on the contact pressure. The maximum contact pressure, P_0, is then given by equation 2.1, which considers the surface as a uniform compressible material:

$$P_0 = \frac{1}{\pi}\sqrt[3]{\frac{6wE'^2}{R^2}} \qquad (2.1)$$

here, w is the normal load and R the radius of a sphere in contact with a plate.
The reduced contact modulus E' is given by:

$$E' = 2\left(\frac{1-\upsilon_1^2}{E_1} + \frac{1-\upsilon_2^2}{E_2}\right)^{-1} \qquad (2.2)$$

here, ν_1 and ν_2 are the Poisson ratios and E_1 and E_2 are the Young's moduli of the two contacting materials 1 and 2.

The deformability of pig's tongue and both soft and hard PDMS was determined by means of measuring the Young´s modulus in compression tests. A major finding, shown in Figure 2.5, was that the elastic modulus of PDMS was 250 times larger than that of pig's tongue.

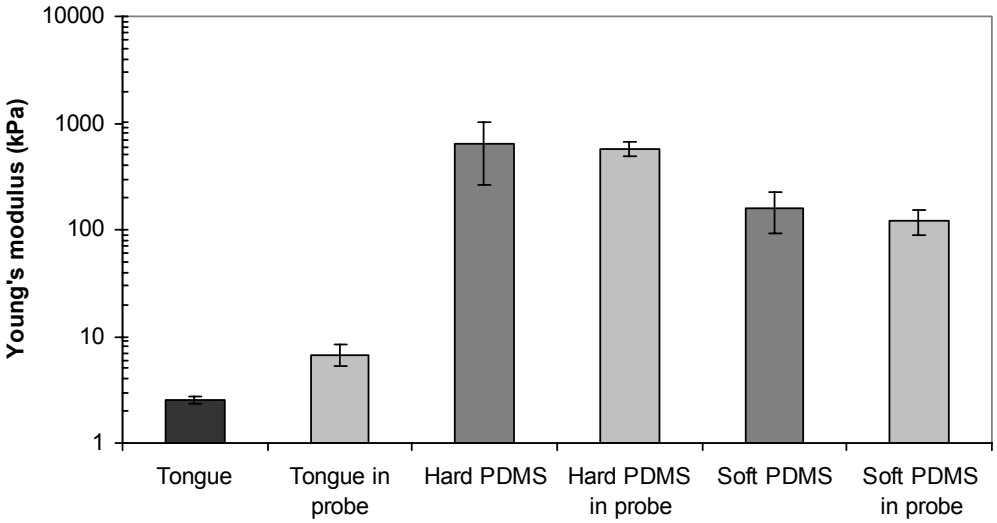

Figure 2.5: Comparison between the elastic modulus of preserved tongue tissue, hard PDMS and soft PDMS and comparison between samples screwed and not screwed into the OTC measuring probe; compression speed 5 mm/s, location on tongue EFGH, frozen according to procedure (§2.3.1).

By using equation 2.1 to estimate the contact pressure for the two types of surfaces at the same normal pressure, it appears that the contact pressure for PDMS is on the order of 30 times higher than for tongue tissue ($\nu_{PDMS} \approx 0.49$ and $\nu_{glass} \approx 0.15$ [29], $\nu_{tongue} \approx 0.49$ [30], $E_{glass} \approx 70$ GPa, $E_{tongue} \approx 2.6$ kPa and $E_{PDMS} \approx 0.65$ MPa (Figure 2.5) and assuming the same R_x for both PDMS and tongue). Although we neglect that equation 2.1 is only applicable to uniform compressible surfaces in point contact, this estimation still indicates that tribology measurements performed with rubber are usually conducted at much higher contact pressure than with tongue tissue. Even the soft PDMS, which contained less cross linker than regular PDMS still had a much higher elastic modulus than tongue tissue.

In order to estimate the Young's modulus of both tongue tissue and PDMS rubber when performing friction measurements with the Optical Tribological Configuration (OTC), the elastic modulus of the surfaces is determined while screwed into the OTC

measuring probe (see Figure 2.3). In Figure 2.5 it can be seen that tongue tissue had a higher elastic compression modulus when confined in a probe. This can be attributed to the fact that the tissue is screwed into the OTC measuring probe which results in pre-stress within the tissue.

A new preserved tongue tissue sample was cut and prepared before each tribology experiment. We therefore determined the influence of the thickness of the sample as well as that of the location on the tongue the sample was taken on the deformability of the surface. Figure 2.6 shows that increasing the thickness of the sample results in a higher Young´s modulus. The tongue consists of an epithelial layer and different muscular layers. Upon compression both the upper and the lower plate deform the sample. In case of the thicker sample, more muscle layers are present in the sample, resulting in a higher elastic modulus. This may be an effect of the presence of a muscle layer with a different fibre orientation. The influence of sample thickness on deformability was not subject to further research. However, this influence does explain why the Young´s moduli of the, 4 times thicker samples given in Table 2.1, were higher than those in Figure 2.5.

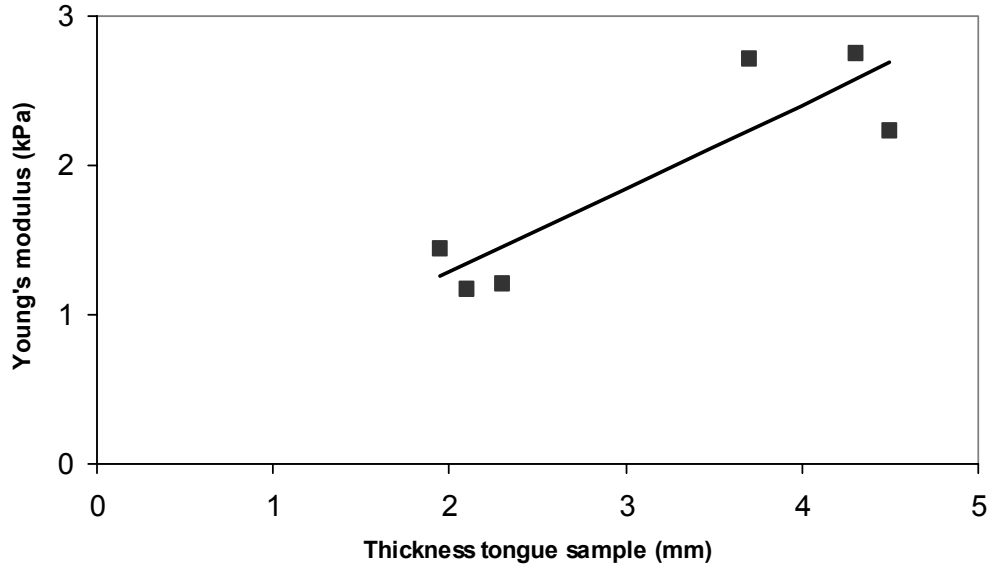

Figure 2.6: Young's modulus as function of the thickness of the tongue samples, compression speed 5 mm/s, sample location EFGH.

Besides the thickness of the samples and the use of the OTC-probe, we also investigated the effect of sample location on the deformability. Figure 2.7 shows that the samples taken from the tip of the tongue, samples A (and B), have a significant lower

elastic modulus than those in the middle of the tongue, samples EHFG. In the OTC this directly influences the contact pressure during the experiments and therefore the experiments are conducted with samples taken at the same place of the tongue only. The tongue is thought to consist of an interwoven 3D-network of skeletal muscle fibres and fibre bundles in which both intrinsic and extrinsic fibres are involved [13; 31]. The fibre orientation differs throughout the tongue [31], which results in a difference in muscular structure from the anterior part to the posterior part.

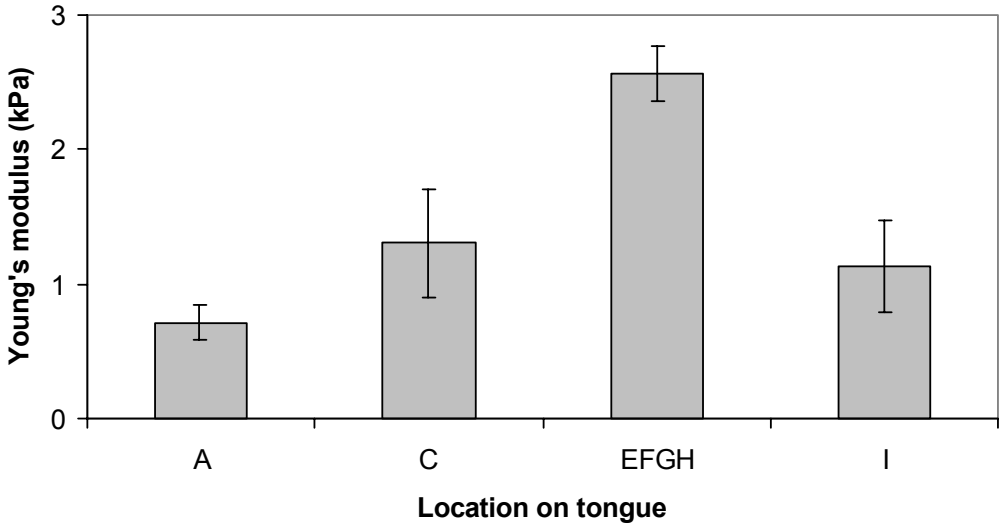

Figure 2.7: Young's modulus as function of location on the tongue, compression speed 5 mm/s

Wetting characteristics

Several studies have been performed to determine the elastic modulus of skin tissue, which displays mechanical characteristics similar to those of mucosal tissue. According to Hendriks et al (2003), skin shows a non-linear stress-strain relationship, time-dependent behaviour, is incompressible, anisotropic and inhomogeneous, and is subjected to a pre-stress. As a consequence, the value of the Young's modulus determined by various methods varies enormously depending on the method (between 1.1 kPa and 20 MPa). The value is also dependent on the length scale of the experiment, as well as on the extent of deformation applied and on the skin thickness [32]. Due to large variation in Young's moduli, specifically depending on the method of determination, it is not possible to compare our results directly with literature values. However, our compression experiments do allow us to compare the deformation behaviour of tongue with PDMS.

Numerous studies relating perception of food to its lubricational behaviour are performed using a rubber as a mimicking surface for the mouth [9; 10]. PDMS is a relative soft rubber, but is still much firmer than tongue tissue (see Figure 2.5), which indicates that in terms of elastic modulus rubber is not a good oral surface mimicking material.

The wetting characteristics of the surfaces were determined by contact angle measurement of water droplets, with sunflower oil as the bulk solution. New preserved tongue and PDMS samples were used for each experiment. Performing contact angle measurements on a very rough surface such as the tongue gives indications on the wetting characteristics of the surface, but due to the roughness can not provide very accurate angles. Even sub-microscopic roughness can lead to hysteresis, which tends to decrease the contact angle measured, whereas for poorly wetted surfaces it tends to increase the angle measured [28].

The tongue samples were thawed shortly before the measurement in tap water of 20 °C, which prevented drying out of the tissue during thawing. Immediately after thawing the samples were dried with tissue paper, resulting in a slightly moist tongue surface. On such a surface, a water droplet was spreading very fast (Figure 2.8a-c), which indicated that under these conditions the tongue surface exhibited hydrophilic behaviour. A tongue sample with a dry surface was obtained by blowing pressurized air over its surface. For such a "dry" surface, contact angle measurements revealed a hydrophobic surface (Figure 2.8d-f). The results show that the tissue of the tongue is intrinsically hydrophobic. In the mouth the surface is covered with a mucus layer of which the mucins (glycoproteins) are thought to be the most important components in lubricating the oral cavity [33]. Mucins are molecules with a high water binding capacity, which adhere easily to various surfaces. The tongue samples used in the contact angle measurements are probably no longer covered with a mucus layer, due to the preparation and preservation of the samples. These results indicate that the tongue deprived of its mucus layer is hydrophobic but the presence of a mucus layer, containing mainly water, can give the tongue a hydrophilic character.

The water/oil contact angle of a dry preserved tongue surface was approximately 115°, whereas for PDMS rubber it was approximately 160º (Figure 2.9). This indicates that PDMS is a more hydrophobic surface than dry tongue tissue.

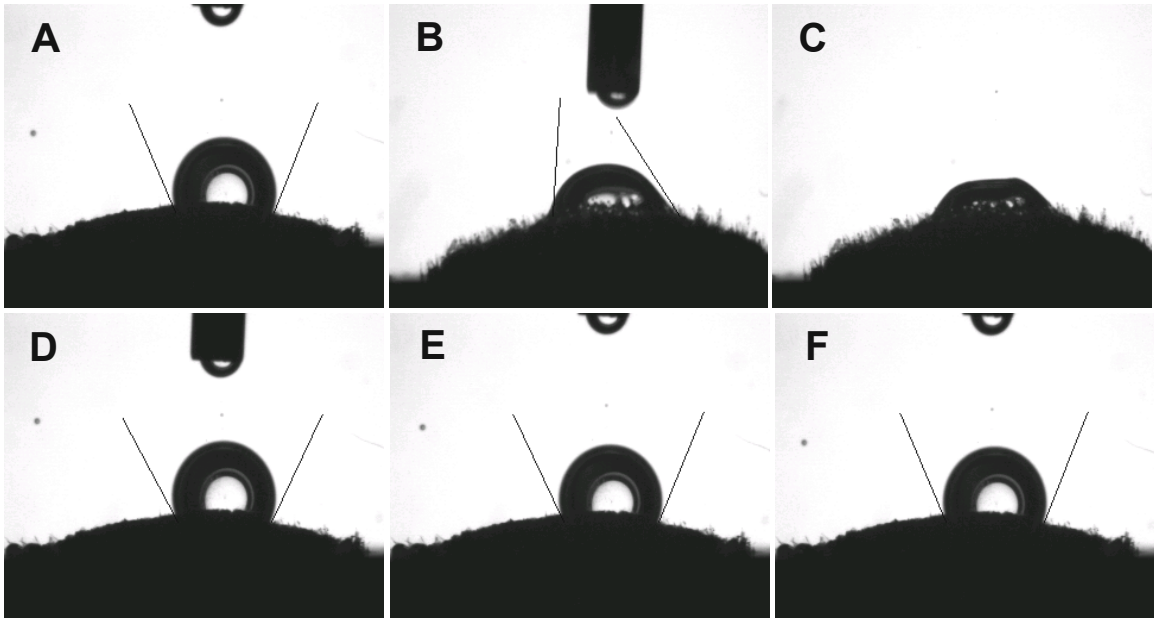

Figure 2.8: Contact angle measurement on pig's tongue, bulk phase sunflower oil, droplet water. a) slightly moist tongue surface with water droplet b) slightly moist tongue after 2 sec c) slightly moist tongue after 5 sec. d) dry tongue surface with water drop e) dry tongue after 1.5 min f) dry tongue after 3 min.

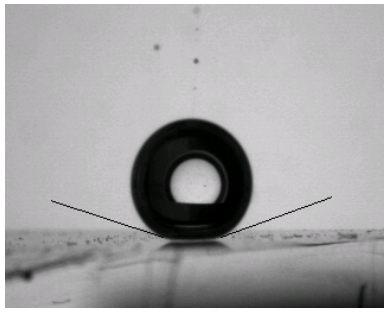

Figure 2.9: Contact angle measurements on flat PDMS, bulk phase sunflower oil, droplet water

Van der Mei et al analysed the water/air contact angles of gingival surface in the in vivo human oral cavity and found contact angles between 70 and 80°. They state that, in general, tissues with adsorptive and exchange functions or in need of lubrication tend to be more hydrophilic, whereas tissues requiring protection against pathogenic microorganisms or acids tend to be hydrophobic [34]. The tongue can be considered as a surface in need of lubrication, but it also requires protection against, e.g., acids. It seems that a hydrophobic surface in combination with coverage by mucins makes it possible to combine both functions.

2.3.3 The effect of surface characteristics on lubrication

Using data for emulsions sheared between steel and rubber, Malone and De Wijk claimed that friction relates to the perception of an emulsion [9; 10]. However, we concluded that the surface characteristics of tongue tissue are rather different from the surface characteristics of rubber. We therefore investigated the effect of this difference on the behaviour of emulsions under shear.

An emulsion (40 wt% oil) was sheared between pig's tongue and glass. CSLM images were taken: A) before applying a load, B) under compression, just after applying the load prior to shear, C) just after shearing still under compression, and D) just after the load had been removed (see §2.2.3). Before applying a load, the emulsion did not contain large droplets, as was verified by means of light scattering.

Figure 2.10B shows that applying a load (emulsion under compression) with tongue tissue as the upper surface, results in coalescence of droplets between the filiform papillae (white hairy structures). The tongue tissue is in contact with the lower glass plate. The contact area is depending on the load applied, the roughness of the sample and the deformability. Thus at some spots there is no gap between the two surfaces and at other spots a gap remains due to the clefts between the papillae. After shearing, oil is released, as can be seen in Figure 2.10C (white arrow). When releasing the pressure, though, the large droplets could not be observed anymore (Figure 2.10D): due to the density difference they cream and move out of the focal plane. Furthermore, Figure 2.10D shows that there are less droplets left in comparison to the situation before shearing, Figure 2.10A, indicating that due to shear-induced coalescence and subsequent oil release, the number of droplets in the focal plain decreased. The images in Figure 2.10 were produced with an emulsion with an average droplet size of 2.4 μm. Similar shear-induced coalescence phenomena were also observed for the emulsion with an average droplet size of 1 μm. In contrast to shearing an emulsion between pig's tongue and glass, we do not observe the occurrence of large droplets shearing the same emulsion between PDMS rubber and glass. This can be concluded from the featureless images of Figure 2.11. This Figure 2.11 does not make clear whether we do not observe larger droplets because they cream out of the focal plane or because coalescence simply does not occur when shearing between glass and rubber. However, comparing number and size of the droplets before

and after shearing with PDMS, supports the idea that shearing with smooth PDMS does indeed not induce coalescence.

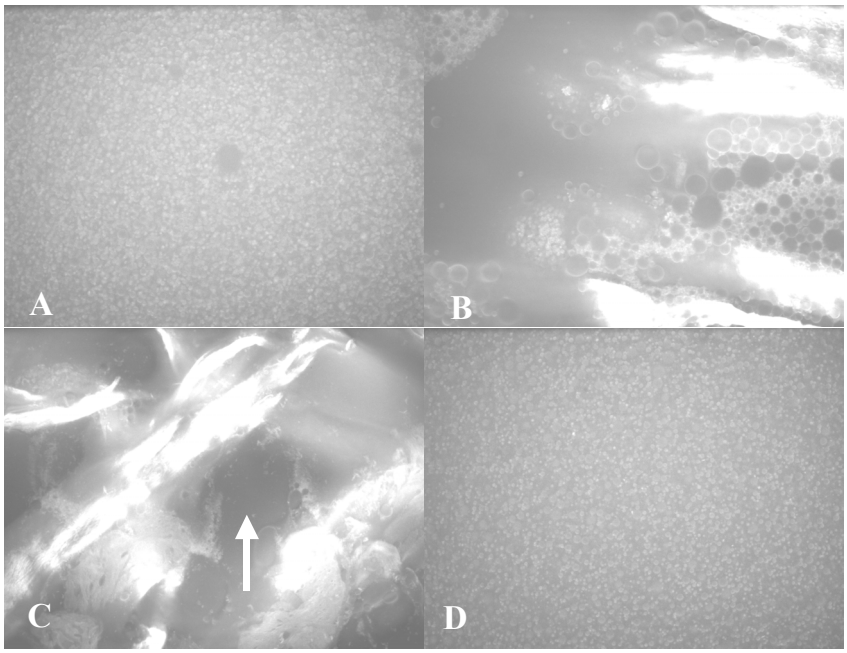

Figure 2.10: CSLM images of a 1 wt% WPI emulsion with 40 wt % sunflower oil sheared between pig's tongue and glass: A) before applying a load B) under compression, just after applying the load, prior to shear C) just after shearing still under compression and D) just after the load was removed. Image size 144 μm x 108 μm, load 0.5 N, speed 80 mm/s.

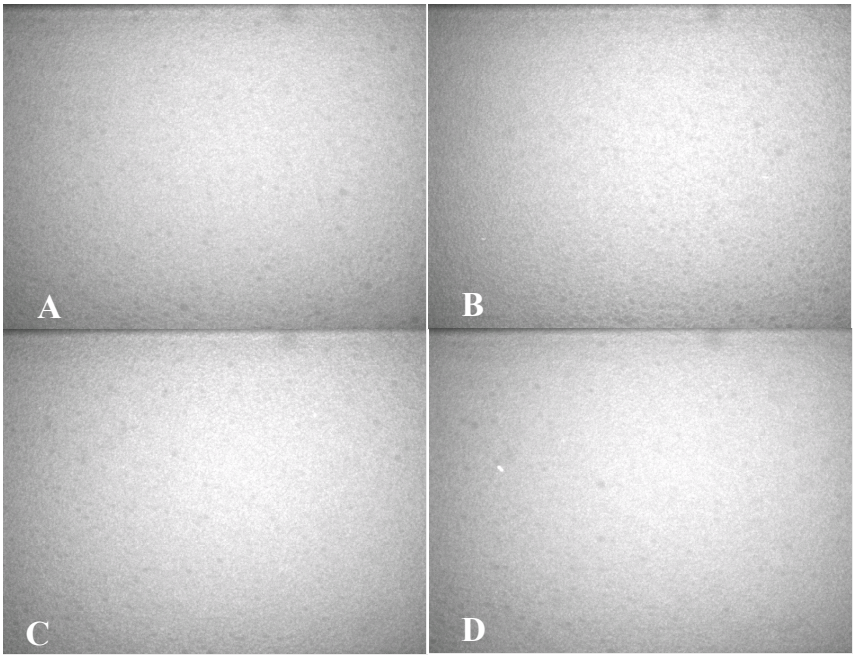

Figure 2.11: CSLM images of a 1 wt% WPI emulsion with 40 wt% sunflower oil sheared between PDMS rubber and glass. A) before applying a load B) after applying a load C) after shearing still under compression and D) after the load was removed . Droplet size, D[3,2], is 1.0μm, image size 144 μm x 108 μm, load 0.5 N, speed 80 mm/s

Thus, the phenomena observed shearing an emulsion between PDMS/glass are completely different from the phenomena occurring when an emulsion is sheared between pig's tongue/glass.

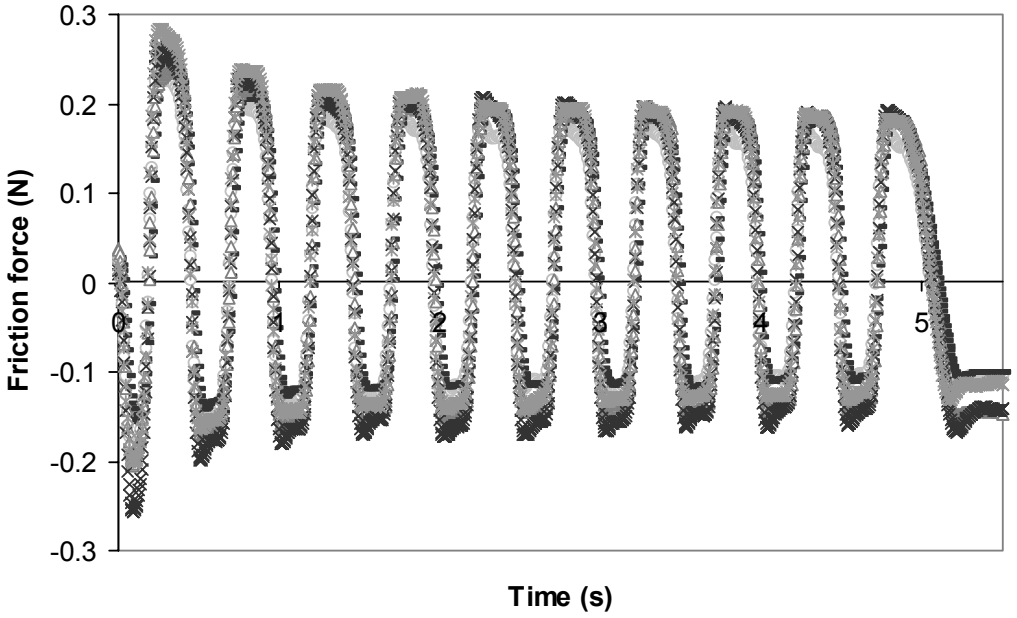

Figure 2.12: Emulsion, 1 wt% WPI, 40 wt% sunflower oil, sheared between tongue and glass; measurement performed 5 times with different pieces of tongue taken at location F.

During shearing of the emulsion between glass and pig's tongue, the friction force was measured, resulting in a typical pattern as presented in Figure 2.12. Figure 2.12 shows that friction forces between tongue and glass with emulsion acting as a lubricant can be measured in a reproducible way, despite the fact that the measurements were conducted with biological material originating from different pig's tongues. The standard error in the friction results obtained was less than 10%. Due to the limited availability and the necessity to repeat the measurement several times, measurements were performed at one speed and one load only (see §2.2.3). The reader may notice that there is a slight inversion asymmetry in the force. This has no physical meaning, but is due to the limitations of the apparatus.

The friction force was also measured when shearing the same emulsion between glass and PDMS. As can be seen in Figure 2.13, the pattern upon shearing is completely different form the pattern in Figure 2.12 for shearing between tongue and glass. The movement starts at the point indicated with the first arrow. Unlike with tongue and glass,

a large static friction (see second arrow) must first be overcome. From the curve in Figure 2.13 it seems that shearing between PDMS and glass gives rise to unidirectional friction. This is due to a slight mobility in the OTC construction, which causes the upper plate (see figure 2.2) not to be in a right angle with the lower plate. The force axes of the force transducer, the unit where the forces are actually measured, are therefore not equal to the real force axes. This causes an underestimation of the true normal force and force offsets in the x-direction and y-direction. In the situation of shearing an emulsion between smooth PDMS and glass the friction force upon moving is very low. The force contribution in the x-direction, due to OTC plates being not completely parallel, is larger than the friction force generated by shearing. This results in an apparent unidirectional friction force. Nevertheless, Figure 2.13 does indicate that tribological behaviour of an emulsion between tongue and glass is very different than between smooth PDMS and glass, the latter giving rise to an estimated friction force of 7 mN (resolution force transducer is 0.78 mN). In the case of shearing between pig's tongue and glass the lubrication regime is the mixed regime, implying that the tissue is partly in contact with the glass. This results in an almost 30 times higher friction force than in case of shearing between smooth PDMS and glass. Shearing between the two smooth surfaces glass and PDMS allows formation of a hydrodynamic film of a lubricant and thus results in very low friction force.

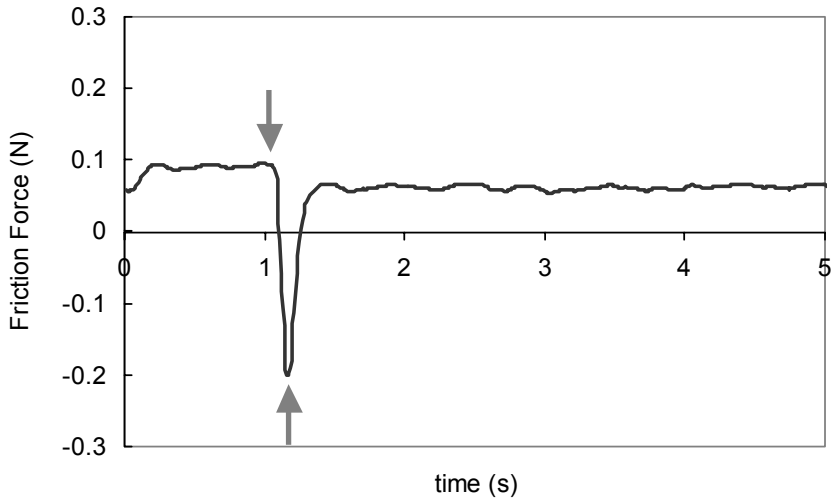

Figure 2.13: Emulsion, 1 wt% WPI and 40 wt% sunflower oil, sheared 10 times between PDMS rubber and glass. The first arrow indicates the start of the movement, the second the static friction force.

In order to understand the phenomena occurring in the mouth and their impact on perception, it is clear that surface characteristics are crucial and should be considered. Knowledge of the essential characteristics of pig's tongue enables us to modify PDMS surfaces in such a way that they will more closely mimic a tongue surface. PDMS is an ideal surface for this since it is easily moulded in various shapes allowing adaptation of the surface roughness. Furthermore, with the plasma treatment already described by Lee et al [17] the wetting characteristics of the PDMS surfaces can be tuned. Most important, PDMS is available in large quantities, it is not subject to large alterations due to retardation and there hardly exist any differences between the PDMS surfaces.

2.4 Conclusion

Friction was reported by many authors to relate to the sensory perception of emulsions. From experimental work performed in this study it is clear that a careful choice of a mouth-representative surface is essential when conducting tribology measurements in relation to oral behaviour of emulsions. Here, we used pig's tongue to mimic the human tongue. The disadvantage of pig's tongue is that it is limited in its availability and it is subject to fast degradation. Moreover, there are individual differences between the pig's tongues implying that many pig's tongues are needed to obtain reproducible results. However, pig's tongue is good reference material for oral tissue.

PDMS is a material often used as a mouth-mimicking surface, which can easily be moulded in various shapes, it can be hydrophilised and it is available in large quantities. Characterisation of both smooth PDMS and pig's tongue on roughness, wetting characteristics and deformability revealed possibilities to modify PDMS in such a way that it can better mimic the tongue.

Pig's tongue is covered by papillae of which especially the filiform papillae give the tongue its roughness. The surface is fairly hydrophobic, but may also act as a hydrophilic surface, when covered by a mucus layer. In comparison with preserved tongue, PDMS has a similar hydrophobicity, but is much smoother and has an elastic modulus, which is of two orders of magnitude larger. As a result of these dissimilarities, different tribological behaviour was observed. In addition, CSLM observation of emulsions under shear during tribological measurements, showed also a difference in

emulsion coalescence behaviour. However, having studied the characteristics of the tongue enables us now to develop PDMS surfaces with similar characteristics that are relevant for tribological behaviour of a tongue.

Acknowledgement

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3

The occurrence of in-mouth coalescence of emulsion droplets in relation to perception of fat

Abstract

We studied the relation between sensitivity of emulsions for in-mouth coalescence and perception of fat-related attributes, such as creaminess, as well as the relation with *in vivo* perceived and *ex vivo* measured friction. Emulsions with varying expected sensitivity towards in-mouth coalescence were engineered, sensorially evaluated using a trained QDA panel and physico-chemically characterized using light scattering and microscopy. Physico-chemical characterization of those *in-vivo* and *ex-vivo* processed emulsions confirmed the expected sensitivity of these systems towards in-mouth coalescence. Experiments showed that both shear-induced and surface-induced coalescence play a role in occurrence of in-mouth coalescence. Furthermore, the emulsions were characterized by performing friction measurements under mouth-mimicking conditions to be able to identify a relation between perceived oral friction and perception of fat-related attributes.

It is shown that the emulsions most sensitive towards in-mouth coalescence gave rise to the highest creamy mouth-feel and fatty sensations as well as oily taste sensation. This indicates that both aroma and mouth-feel are of importance in fat perception. Combining friction force measurements with sensory analyses indicated that occurrence of coalescence gives rise to an enhanced fat perception and also to a lowering of the orally perceived and experimentally measured friction. The results shown open the way to manufacture reduced fat emulsions with a full fat sensation.

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3.1 Introduction

Designing food emulsions that contain less fat, but nevertheless give a full-fat in-mouth sensation is of great commercial and public interest. Food industry and food science have therefore a joint interest in understanding and controlling the perception of fat-related sensations of products. The perception of a food product mainly takes place in the mouth. Hence, understanding in-mouth behaviour of food emulsion droplets will contribute to more knowledge on effectively engineering emulsions with a desired fat sensation.

In Chapter 2 it was shown that coalescence of emulsion droplets upon oral processing and possibly also adherence of emulsion components to the oral mucosa occurs. These two in-mouth phenomena may be of importance for in-mouth perception. In this study we focus on the relation between occurrence of in-mouth coalescence and perception.

Coalescence can occur through various processes. In the context of the mouth, we expect that shear-induced, surface-induced, saliva-induced and air-induced coalescence play a role. Shear increases the frequency of droplets encountering each other and therefore enhances the sensitivity to coalescence [1]. Coalescence can also be surface-induced meaning that droplets spread on a solid surface. Driving force for this is the interfacial tension difference between the solid, the gas and the liquid phase [2]. The occurrence of surface-induced coalescence in the mouth is therefore expected to be related to the wetting characteristics of the oral mucosa as well as the characteristics of the interfacial layer stabilizing the emulsion droplet (see Chapter 2). Air-induced coalescence can be a third cause for coalescence in the mouth [3]. Upon mastication, air is included in the food matrix causing the droplets to spread on the air/water interface, similar to the process described for whipping of cream by Hotrum et al [4]. Saliva-induced coalescence occurs in emulsions stabilized by starch-based emulsifiers due to the activity of amylase, an enzyme present in saliva.

In this study we investigated the occurrence of in-mouth coalescence and its implication on sensory perception. To the best of our knowledge no study has been reported previously on this subject.

The approach taken here was to develop emulsions with an expected difference in sensitivity towards coalescence. This sensitivity was varied by engineering emulsions with a variation in type of emulsifier, amount of emulsifier, droplet size and fat type. By reducing the amount of emulsifier to such an extent that the oil/water interface is scarcely covered, one can increase the susceptibility of emulsions to shear-induced coalescence. Other ways to increase the susceptibility to shear-induced coalescence are to increase the droplet size and to include solid fat in the emulsions. Fat crystals in the oil phase can promote partial coalescence [5], which upon melting of the crystals in the mouth results in an increased droplet size. Furthermore, an emulsion with an expected high stability to shear-induced coalescence was obtained by stabilizing the droplets by both whey protein isolate and sodium-caseinate and heating it after homogenisation to increase the interfacial layer thickness [6]. Emulsions sensitive to saliva-induced coalescence were developed by using a starch-based emulsifier (patent application EP06117370.4). The relation between sensitivity towards coalescence and perception was determined by sensorial evaluation of all these emulsions.

Simultaneously with the identification of the role of coalescence in perception, we measured the variation in occurrence of coalescence of the different emulsions using several techniques. Subsequently, the results of the sensorial experiments are correlated with *in-vivo* and *ex-vivo* observations of the occurrence of coalescence. It has been reported that perception of fat-related attributes, such as creaminess, correlate with friction forces sensed between tongue and palate [7; 8]. The relation with coalescence could be that enlarged oil droplets affect the lubricating layer properties by forming a fatty coating on the oral mucosa, which causes a reduction in sensed friction force. Another way in which coalescence might influence fat-perception is by affecting aroma release. Aroma perception is suggested to correlate with perception of creaminess [9-11]. However, the relation between aroma release and perception of fat-related attributes was beyond the scope of this study.

In order to avoid confusion about the terms aroma we used the terminology proposed by de Roos [12], who regards flavour as a combined effect of odor (or aroma), taste and mouth-feel.

3.2 Material and Methods

3.2.1 Emulsion preparation and characterization

Emulsions with expected differences in sensitivity to coalescence were produced according to Table 3.1. The emulsifiers were dissolved overnight at 4°C in double distilled demineralised water containing 0.02 wt% sucrose (Suikerunie, Groningen, Netherlands), 0.01 M NaCl (NeZo, Akzo Nobel, Amersfoort, Netherlands) and 0.033 wt% vanilla aroma (T03912, Danisco, Denmark). The pH of the protein solutions was adjusted at 4°C to pH 6.7 by adding 0.1 M of HCl or NaOH (Sigma Aldrich, Steinheim, Germany). Pre-emulsions containing 40 wt% fat phase and the different emulsifiers were made using the ultraturrax (T25, IKA, Switzerland) for 1 min. All the emulsions were homogenised at 50°C with a Rannie homogeniser (APV, Denmark) at 70 bar to obtain small droplets and at 40 bar for larger droplets. The emulsions made 10 passes through the homogeniser. After homogenisation the emulsions were kept on ice for several hours before storing them at 4°C. After 1 night of storage at 4°C the protein-stabilized emulsions were diluted with the continuous phase containing emulsifier, sucrose, NaCl and vanilla aroma, to obtain a 10 wt% fat phase emulsion.

Table 3.1: Emulsions differing in fat phase (sunflower oil (SF) vs. Palm Fat (PF)), droplet size (small vs. large), amount of emulsifier (1 wt% vs. 0.1-0.3 wt%) and kind of emulsifier (WPI vs. OSA vs. WPI/NC). Expected sensitivity to coalescence ranged between (1) very stable against shear, (2) stable, (3) unstable and (4) unstable against amylase.

	Fat Phase			
Emulsifier	**Sunflower oil (SF)**		**Palm fat (PF)**	
	Small (S)	Large (L)	Small (S)	Large (L)
WPI/NC	1	1	-	1
WPI 1 wt%	2	2	3	3
WPI 0.1, 0.2 & 0.3 wt%	3	3	3	3
OSA	4	4	-	-

"Very stable" emulsions were produced by using a mixture of 0.95 wt% Whey Protein Isolate, WPI, (BiPro, Davisco, USA) and 0.05 wt% Na-Caseinate, NC (DMV, Veghel, The Netherlands) as emulsifier. Both sunflower oil (fully winterized sunflower oil, a gift by Cargill Amsterdam, Netherlands) and palm fat (Effekta Special, 100% liquid

at T=37°C, a gift by Loders Croklaan, Rotterdam, Netherlands) were used as fat phase. After homogenization and cooling the emulsions on ice, the WPI/NC emulsions were heated for 17 min in a water bath of 90°C.

The "stable" emulsions were stabilized by WPI and contained sunflower oil as fat phase. The "unstable" emulsions were engineered by using palm fat as the fat phase and/or using less emulsifier. To obtain emulsions stabilized with low amounts of WPI but with comparable protein surface coverage the concentration of WPI was varied with the droplet size: 0.3 wt% WPI for small droplets and 0.1 wt% WPI for large droplets (0.2 wt% WPI when solid fat was used).

"Amylase-sensitive" emulsions were produced using octenylsuccinate starch, OSA (Cleargum CO 01, Roquette, Lestrum France) as emulsifier. These emulsions contained 10 wt% sunflower oil upon homogenization and were not diluted. The pH of these emulsions was adjusted after homogenization.

The droplet size distribution was determined by light scattering (Mastersizer 2000, Malvern, Worchestershire, UK). Rheological characterization was performed at 20°C with a Physica MCR 300 (Anton Paar, Graz, Austria) using a cone-plate geometry with a diameter of 75 mm and a 1º angle. The possible presence of clusters and aggregates was determined by light microscopy (Olympus BX60, Olympus Optical CO., Hamburg, Germany). The physical-chemical characterisation of the emulsions was performed at the same day of the sensorial evaluation.

3.2.2 Sensorial analyses

The emulsions were evaluated by a sensory trained panel according to the principles of Quantitative Descriptive Analysis [13]. The panel consisted of 8 female panellists with a mean age of 45.5 years (st. dev 9.4 years, max. 59 years and min. 33 years). All subjects had previously been screened to exclude olfactory and taste disorders and received training in the description of odour, taste, mouth-feel, and after-feel attributes. The panellists were unaware of the research question and did not have a scientific background. The subjects were paid for their participation. Testing took place at the sensory facilities of the centre of Innovative Consumer Studies, Agrotechnology and Food Science Group, Wageningen UR, The Netherlands.

The panellists generated in separate sessions descriptive attributes of which 38 were used to profile the 13 different emulsions (see appendix). The trained panel received 1 extra specific training session to familiarize with the emulsions to be examined. The panellists were seated in climate-controlled sensory booths and the acquisition was done by computer using FIZZ software (Biosystemes 1998, v1.20K, Couternon, France).

The panelists judged the set of emulsions in a semi-monadically assessment procedure in triplicate on visual analogue scales. The emulsions were served at room temperature semi-monadically in a presentation order, which was randomly designed over panellists (balanced latin square design), starting with a warm-up sample (excluded from the data analysis). The thirteen emulsions were tasted in four blocks with short breaks in between (5-10 minutes). The emulsions were evaluated on 4 odour (O), 10 taste (T), 9 mouth-feel (M), 4 after-taste (AT) and 11 after-feel (AF) attributes (see appendix). The panellists smelled the product upon opening of the cup, and then took at least two sips from the product. After evaluation of taste and mouth-feel attributes, the product was spat out and after-taste and after-feel were judged. Between two different products the panellists cleaned their mouth with white bread and soured water (0.2 g/L citric acid). In addition, cream crackers and tap water could be taken ad libitum.

Sensory data were analysed by general linear model ANOVA with LSD (least significant difference) analysis as post-analysis. When appropriate, repeated measures ANOVA analyses were conducted using the statistical analyses software SPSS (SPSS, SPSS Inc., Chicago). In addition, a Principle Component Analyses (PCA, Unscrambler, Camo Inc.) was performed.

3.2.3 Physico-chemical analyses on occurrence of coalescence

To study the occurrence of coalescence and its effect on lubrication the emulsions were characterized by different methods on the same day as the sensorial evaluation.

Tribological measurements

The emulsions were sheared in the Optical Tribological Configuration (OTC) according to the method described in Chapter 2. In short, emulsions were sheared between a piece of the dorsal surface of a pig's tongue and glass, while friction forces were measured simultaneously. The speed of shearing was 80 mm/s and the load applied was

0.5 N. Subsequently, the sheared emulsions were gently rinsed off the tongue samples with 5 ml of demineralised water using a 5 ml pipette, avoiding further coalescence. The same procedure was applied to the glass plate. The rinse samples were evaluated separately. For comparison, the emulsions were also brought into contact with pig's tongue and glass, without applying the shear treatment, and subsequently rinsed off according to the same procedure. The droplet size distribution (Mastersizer 2000) of the rinse samples was determined; the distribution after shearing in the OTC was compared to the one before shearing and the one after being in contact with pig's tongue or glass. All experiments were conducted in triplicate, using a new pig's tongue sample every time, and performed at room temperature in absence of saliva.

Oral processing

Six persons took 10 gram of the emulsions in their mouth, processed them by moving the tongue up and down during 30 seconds and subsequently spat out the residue in 18 grams of water. The OSA samples were spat out in 0.06% SDS solution in order to prevent the droplets to coalesce further. The effect of oral processing on the droplet size distribution was analysed using light microscopy (Olympus BX60) and light scattering (Mastersizer 2000). In this experiment only the emulsions with larger droplets were used.

3.3 Results

The trained panel sensorially evaluated thirteen different emulsions with expected variations in sensitivity towards coalescence in the mouth (see Table 3.1) on 35 sensory attributes (see appendix). The same emulsions were analysed on sensitivity towards coalescence by performing physico-chemical measurements.

3.3.1 Sensory results

The average scores of the different attributes and products are listed in Table 3.2. Table 3.2 clearly shows that the OSA-stabilised emulsions, which are expected to be least stable in the mouth, received significant higher scores on fat-related taste, mouth-feel and after-feel attributes such as Toil, M/AFcreamy, M/AFfatty and Mthick. On the other hand, these unstable (against amylase) emulsions received significant lower scores on oral-friction related attributes such as AFrawtongue, AFcoating and AFrough. This confirms

our expectations that emulsions more sensitive to coalescence are perceived as lowest in oral friction and receive the highest scores on fat-related attributes. In other words, this indicates that fat perception is inversely related to oral perceived friction.

Table 3.2 also shows that the large sensory effect of the amylase unstable OSA emulsions tends to overshadow the possible differences between the other emulsions studied. The differences between the other emulsions are therefore less pronounced. For this reason we clustered the rated sensorial intensities of the emulsions based on expected difference in sensitivity towards coalescence as indicated in Table 3.1. The results include emulsions differing in droplet size and fat phase. In Figure 3.1 we only discuss attributes, which are expected to relate or inversely relate to fat-perception. Also, repeated ANOVA measures were performed on mouth- and after-feel attributes for the stable (1% WPI) and unstable (0.1-0.3% WPI) emulsions, in order to determine what the influence of the emulsifier concentration is, whether particle size matters and what the influence is of using Palm fat (PF) instead of sunflower oil (SF).

Clustering of the sensory scores shows again that the OSA-stabilised emulsions received the highest fat-related scores on mouth-feel and after-feel attributes and were scored lowest on friction (Figure 3.1). Note that the OSA emulsions also received the highest scores on taste intensity, taste oil and taste vanilla. The taste of vanilla is thought to be associated by most individuals with creaminess, since vanilla is often used in high fat creamy products. On the other hand, the difference in perception of fat-related attributes between the stable (1% WPI) and unstable (0.1% WPI) emulsions is for most attributes not significant. Despite this, we do observe a trend, meaning that the sensory ratings of most fat-related attributes (e.g. Mfatty, Mthick, Mslippery, AFcreamy, Toil, Tintensity and Tvanilla) were scored higher for the unstable emulsions compared to the stable emulsions. In addition, the very stable emulsion was always perceived as more rough (e.g. Mdry, AFrough, AFraw tongue and AF astringent) than the unstable emulsions. So in short, the expected ranking in sensitivity towards coalescence was found to correspond to an, not always significant, increase in the rated sensorial intensity, of the attributes mouth-feel creamy, fatty, thick, slippery and after-feel creamy and coating, whereas a decrease was found for the attributes mouth-feel dry and after-feel rough, raw tongue and astringent.

Table 3.2: Mean sensory scores for emulsion ranging from very stable until unstable against amylase and ANOVA analyses

	very stable	very stable	(very) stable	stable	stable	unstable	unstable	unstable	unstable	unstable	unstable	unstable against amylase	unstable against amylase	Significance
	1% WPI/NC SF S	1% WPI/NC SF L	1% WPI/NC PF S	1% WPI SF S	1% WPI SF L	1% WPI PF S	1% WPI PF L	0.3% WPI SF S	0.1% WPI SF S	0.3% WPI PF S	0.2% WPI PF L	2.5% OSA SF S	2.5% OSA SF L	
Odour attributes														
Ointensity	51.57	48.52	40.38	36.05	39.62	41.52	41.9	40.71	50.71	45.81	47.67	52.9	43.81	
	AB	ABCD	CDE	E	DE	BCDE	BCDE	CDE	ABC	ABCDE	ABCD	A	ABCDE	*
Ovanilla	36.38	33.14	26.76	33.1	35.05	40.62	33.9	35.86	34.62	35.33	35.95	36.57	38.48	
	!	!	!	!	!	!	!	!	!	!	!	!	!	
Ochalk	35.05	23.1	20.24	17	20.81	22.24	20.57	24.95	37.48	23.86	34.95	36.52	35.1	
	A	B	B	B	B	B	B	B	A	B	A	A	A	***
Taste attributes														
Tintensity	64.76	60.33	48.9	52.86	54.38	65.29	60	56.9	65.57	64.48	68.57	83.57	83.43	
	BC	BCD	E	DE	DE	BC	BCD	CDE	BC	BC	B	A	A	***
Tvanilla	40.38	39.14	29.81	40.48	38.71	48	48.29	39.81	46.14	45.57	54.43	57.76	61.95	
	D	DE	E	D	DE	BCD	BCD	D	CD	CD	ABC	AB	A	***
Tsweet	42.38	43.1	37.95	50.24	44	50.95	50.24	43.19	50.33	46.86	52.9	62.1	65.71	
	CD	CD	D	BC	CD	BC	BC	CD	BC	BC	B	A	A	***
Tsour	16.33	19	22.1	20.14	18.95	14.52	20.52	16.29	16.33	19.71	17.19	16.14	12.33	
	!	!	!	!	!	!	!	!	!	!	!	!	!	
Tsalty	27.19	23.38	14.29	20.05	19.14	23.05	23.57	27.81	26.62	27.52	28.43	31.86	27.52	
	AB	BCD	E	CDE	DE	BCD	BCD	AB	ABC	AB	AB	A	AB	***
Toil	35.86	24.14	17.33	25.86	23.67	27.76	33.38	27.33	34.1	37.86	34.38	51.71	46.62	
	BC	EF	F	DEF	EF	CDE	BCD	CDE	BCD	B	BCD	A	A	***
Tartificialcream	38.24	29.95	30.86	34.52	30.81	41.05	44.95	42.52	42.86	45.14	46.48	58	60.19	
	BCD	D	D	CD	D	BC	B	BC	BC	B	B	A	A	***
Tglue	20.52	18.86	12.62	14.81	17.52	17.81	21.95	18.9	20.43	17.86	18.1	18.81	11.43	
	!	!	!	!	!	!	!	!	!	!	!	!	!	
Mouth-feel attributes														
Mthick	34	38	31.57	34.1	29.9	31.76	37.24	30.67	37.38	35.81	40.38	59.48	67.57	
	BC	BC	BC	BC	C	BC	BC	C	BC	BC	B	A	A	***
Mcreamy	44	41.48	37.76	39.43	37.67	39.81	48.29	38.52	46	46.48	54.05	75.14	73.57	
	CDE	CDE	DE	DE	E	CDE	BC	DE	BCDE	BCD	B	A	A	***
Mfatty	40.52	36.24	33.95	34.52	31.52	36.1	43.48	39.52	46.86	43.38	52.24	74.24	71.71	
	CDE	DEF	EF	EF	F	DEF	CD	CDEF	BC	CD	B	A	A	***
Mslippery	54.43	53.57	49.52	52.38	51	51.1	57.67	53.86	56.71	55.38	54.05	73.71	71	
	B	B	B	B	B	B	B	B	B	B	B	A	A	***
Msticky	27.1	27.24	28.24	27.38	29.76	30.24	27.48	28.57	29.67	27.71	29.57	35.71	33.24	
	C	C	BC	C	BC	BC	C	BC	BC	C	BC	A	AB	*
Mrough	33.43	34.14	29.48	32	32.95	28.81	29.33	29.29	30.76	31.76	30.67	24.76	27.14	
	!	!	!	!	!	!	!	!	!	!	!	!	!	
Mdry	37	35.05	34.86	30.71	32.33	30.1	24.1	29.67	31.86	32.05	24.48	21.1	25.57	
	A	AB	AB	ABCD	ABC	ABCD	DE	BCD	ABC	ABC	DE	E	CDE	***
Mmelting	12.24	13.81	10.19	10.14	13.29	11.95	13	12.81	11.29	16	12.86	15.62	17.62	
	BCD	ABCD	D	D	ABCD	BCD	BCD	BCD	CD	AB	BCD	ABC	A	*
Mmouthfilling	40.05	38.81	37.33	39.05	35.71	46.1	44.43	38.48	46.38	48.9	52.1	72.52	77.24	
	CDE	CDE	DE	CDE	E	BC	BCD	CDE	BC	B	B	A	A	***

Table 3.2 (continued)

After-taste attributes														
ATsweet	38.52	36.19	39.14	45.62	43.19	43.43	43.52	39.38	44.19	42.57	50.57	63.9	65.76	
	CD	D	CD	BC	BCD	BCD	BCD	CD	BCD	BCD	B	A	A	***
ATbitter	35.29	30.33	21.43	27	28.24	30.14	28.38	31.24	28.71	28.19	26.57	16.95	14.52	
	A	AB	BC	AB	AB	AB	AB	A	AB	AB	AB	C	C	***
ATmetal like	41.76	35.14	26.24	27.95	31.95	30.86	24.52	36.14	25	26.52	23.14	24.33	21.81	
	A	ABC	CDE	BCDE	BCD	BCDE	DE	AB	DE	CDE	DE	DE	E	***
ATduration	55.95	51.95	45.76	44.95	47.67	54.62	48.43	52.05	56.52	55.67	56.19	70.86	76.86	
	BC	BCD	D	D	CD	BC	BCD	BCD	B	BC	B	A	A	***
After-feel attributes														
AFastringent	25.86	27	24.52	26.29	25.67	21.43	16.81	24.95	25.95	24.14	20.62	17.48	13.33	
	A	A	AB	A	A	ABC	CD	A	A	ABC	ABCD	BCD	D	**
AFcreamy	29.48	26	21.29	31.05	26.67	32.48	33.48	26.9	32.71	35.95	45.38	68.76	72.81	
	CD	DE	E	CD	DE	CD	CD	DE	CD	C	B	A	A	***
AFdry	45.76	50.57	48.48	44.19	46.57	38.57	39.1	44.52	41.1	41.57	38.43	26.95	27.52	
	ABC	A	AB	ABC	ABC	C	C	ABC	BC	BC	C	D	D	***
AFrough	41.81	46.67	43.14	39.19	36.76	35.1	35.24	43.29	39.62	36.43	35.38	29.67	27.14	
	AB	A	AB	AB	BC	BCD	BCD	AB	AB	BC	BCD	CD	D	***
AFraw tongue	30.52	30.24	34.76	30.52	30.24	20.62	29.19	30.9	25.38	28.33	27	16.67	22.14	
	ABC	ABC	A	ABC	ABC	DE	ABCD	AB	BCD	ABCD	ABCD	E	CDE	**
AFmealy	14.95	17.9	15.33	14.95	17.9	15	19.52	20.05	16.19	17.43	19.38	15.19	14.24	
	!	!	!	!	!	!	!	!	!	!	!	!	!	
AFslimy	24.43	25.48	20.33	24.43	25.48	25.62	28.52	24.62	30.1	27.9	37.24	50.19	47.1	
	CD	CD	D	CD	CD	CD	CD	CD	BC	CD	B	A	A	***
AFcoating	39	41.1	34.86	39	41.1	39.33	43	41.76	43.24	39.1	51.52	68.33	71.05	
	CD	CD	CD	CD	CD	CD	BCD	CD	BC	CD	B	A	A	***
AFsticky	27.95	29.76	26	27.95	29.76	29.86	29.67	30.52	30.19	31.24	29.52	42.76	42.24	
	B	B	B	B	B	B	B	B	B	B	B	A	A	***
AFsatiation	28.14	30.76	23.67	28.14	30.76	37.95	36.57	31.1	35.76	41.71	47.67	70.81	74.86	
	FG	DEFG	G	FG	DEFG	CD	CDE	DEFG	CDEF	BC	B	A	A	***
AFtingling	20.1	13.38	11.52	20.1	13.38	12.33	13.38	14.1	14.19	15.9	14.33	12.86	10.19	
	!	!	!	!	!	!	!	!	!	!	!	!	!	

Significant differences indicated with asterisk: * $p<0.05$, ** $p<0.01$, *** $p< 0.001$

Different letters indicate a significant difference at $p< 0.05$; ! = not significant

The error bars in Figure 3.1 show that the difference in perception between the (very) stable and unstable emulsions is small and overshadowed by the OSA emulsions. An ANOVA was performed to be able to compare the stable (1% WPI) and unstable (0.1% WPI) emulsions. Table 3.3 shows that as a function of percentage emulsifier significant differences are found for Mthick, Mcreamy, Mfatty, Mmouthfilling, AFcreamy and AFsatiation. This shows indeed that the less stable emulsion (0.1% WPI) is perceived as more fatty. Also the fat phase had an effect on the perception of fat-related attributes: palm fat emulsions received significantly higher scores on Mfatty, AFcreamy, and significant lower scores on the oral-friction related attributes AFdry and AFrough. The difference in particle size applied here hardly affected fat perception.

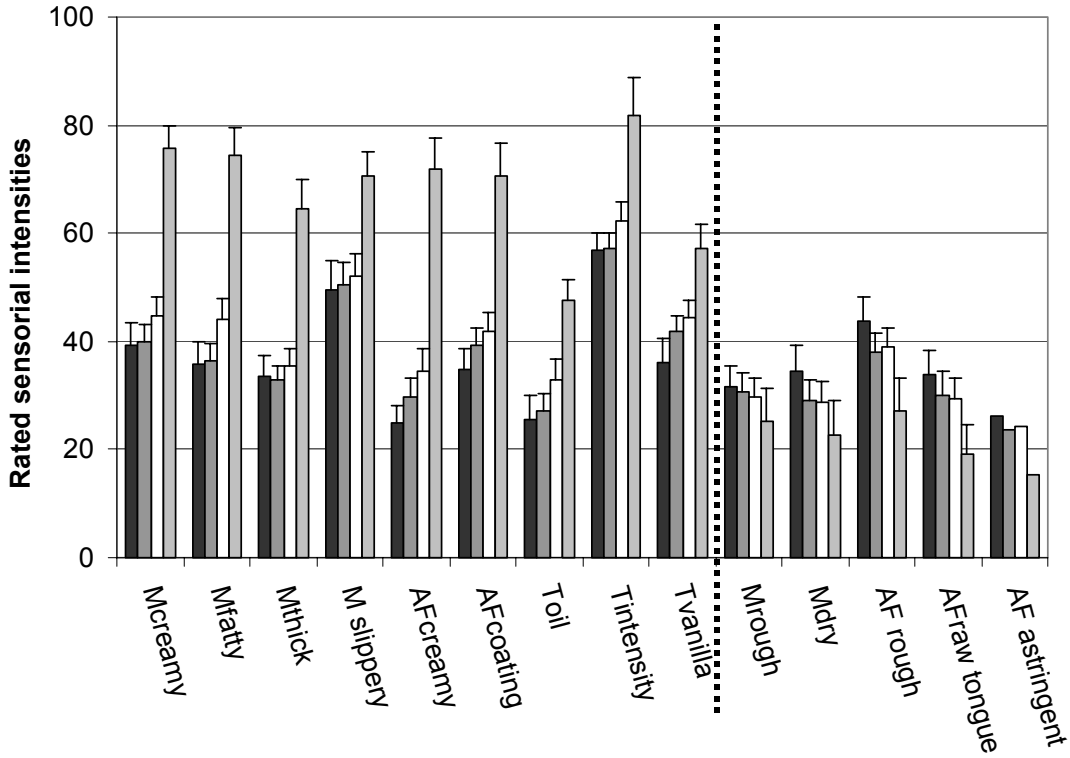

Figure 3.1: Average rated sensorial intensities of emulsions stabilized by 1 wt% WPI/NC ■, 1 wt% WPI ■, 0.1/0.3 wt% WPI □ and OSA ■. Results on emulsions containing solid fat and sunflower oil are clustered. Error bar indicates calculated standard error.

In conclusion, emulsions expected to be more sensitive towards in-mouth coalescence are perceived as more creamy, fatty and also receive low scores on perceived oral friction.

Table 3.3: Repeated measures ANOVA on mouth- and after-feel attributes for stable (1%WPI) and unstable (0.1-0.3% WPI) emulsions

	Effect % emulsifier	Effect SF/PF	Effect particle size
Mthick	*		
Mcreamy	*		
Mfatty	*	*	
Mslippery			
Msticky			
Mrough			
Mdry			
Mmelting			
Mmouthfilling	*	*	
AFastringent		***	
AFcreamy	*	*	*
AFdry		*	
AFrough		*	
AFraw tongue			
AFmealy			
AFslimy		*	
AFcoating			
AFsticky			
AFsatiation	*	*	
AFtingling			*

*Significant differences indicated with asterisks: *P<0.05, *** P<0.001*

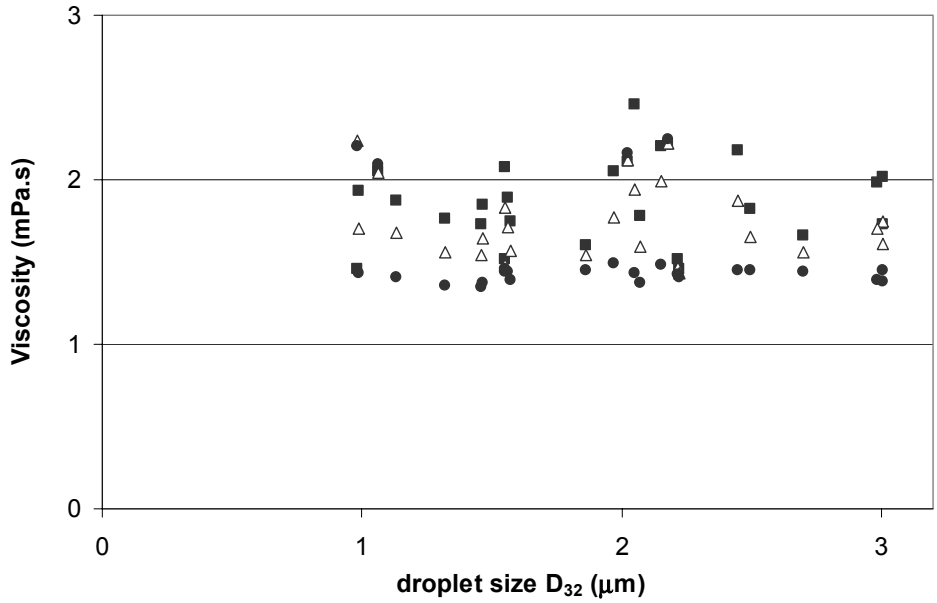

Figure 3.2: Viscosity as function of the average droplet size, D_{32}, of the thirteen different emulsions in two profiling sessions, given at a shear rate of 50 s^{-1} (■),90 s^{-1} (△) and 1000 s^{-1} (●)

3.3.2 Emulsion characteristics

All emulsions were characterised with light microscopy, light scattering and rheology. No aggregates were observed with light microscopy (data not shown). Based on the light scattering data the emulsions were divided into two groups; a group with droplet sizes ranging from 1 µm until 1.9 µm, indicated as 'small' and a group with sizes ranging from 2 µm until 3 µm indicated as 'large'.

In our sensory data we observed that with increasing perception of 'creaminess' also the perception of 'thickness' increased. Furthermore, the perception of 'thickness' was much higher for the OSA-emulsions than for the other emulsions. However, Figure 3.2 shows that the viscosity of all samples was very similar, implying that the sensed difference in 'thickness' was not due to a difference in initial viscosity of the samples.

3.3.3 Physico-chemical analyses on occurrence of coalescence

Partial coalescence upon shearing in a rheometer

Figure 3.3 shows an increase in viscosity in shear rate sweep experiments conducted with emulsions containing solid fat, whereas no increase was observed with emulsions containing sunflower oil. Probably due to the presence of fat crystals, the emulsion droplets clustered together resulting in larger structures (partial coalescence), which raised the viscosity. The resulting increase in viscosity was observed in all systems containing solid fat, including the systems containing small droplets.

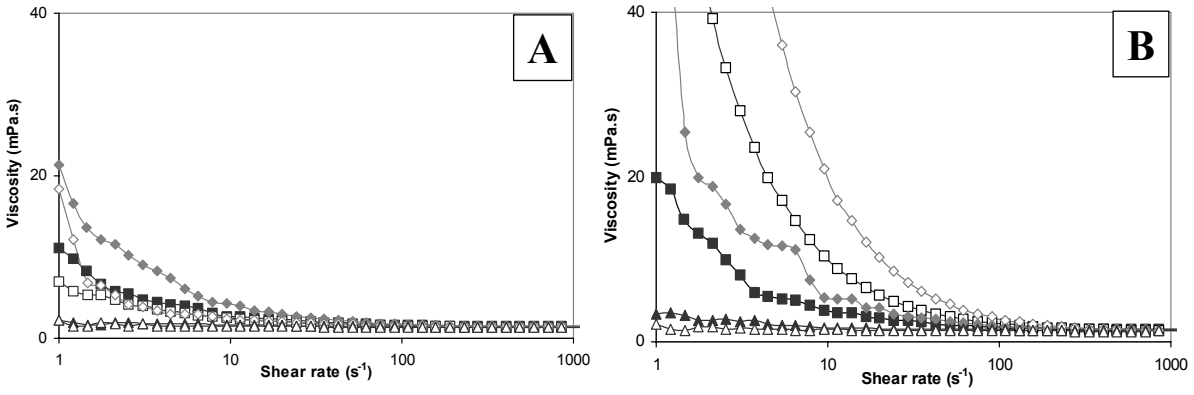

Figure 3.3: Viscosity as a function of shear rate for emulsions with large droplets containing sunflower oil A) and palm fat B) stabilized by: 1wt % WPI (■), 0.1 wt% WPI (♦) or 1 wt% WPI/NC (▲). Closed symbols: increasing shear rate 1-1500 s^{-1}; open symbols for decreasing shear rate 1500-1 s^{-1}.

Coalescence upon shearing in the OTC

The emulsions were sheared in the Optical Tribological Configuration (OTC). To determine the sensitivity of the emulsions towards coalescence, the average droplet size was determined before shearing, after contact with pig's tongue, after contact with glass and after shearing between pig's tongue and glass.

Figure 3.4 shows that the average droplet size of emulsions with initial small droplets increased after being in contact with the tongue surface, except for the emulsion expected to be stable to coalescence, 1 wt% WPI SF and 1 wt% WPI/NC SF. In all cases the droplet size increased even further when the emulsions were sheared between tongue and glass. The largest increase in droplet size occurred for emulsions with a higher expected sensitivity towards coalescence (see Table 3.1). The same trend was found in emulsions with large droplets, although the apparent relative increase in droplet size was less. This could be explained by the limitations of the measuring technique as explained below.

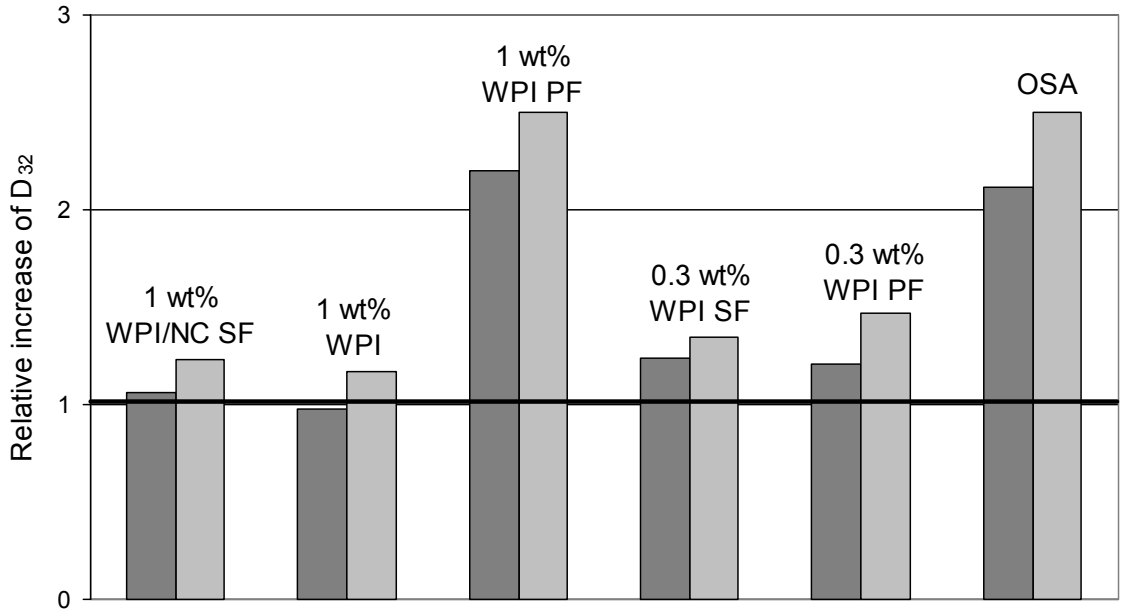

Figure 3.4: The relative increase of emulsion droplet size, D_{32}, of the droplets with respect to the initial droplet size 1) in contact with the tongue surface (dark grey columns) and 2) both in contact with the tongue and sheared between tongue and glass (light grey columns). The emulsions contained small droplets. The fat phase consisted of Sunflower oil (SF) or palm fat (PF). No saliva was present.

Large droplets are difficult to disperse in water because they tend to cream to the surface. Therefore, emulsion droplets larger then around 50 µm were not detected by light

scattering. Figure 3.5 shows that when droplets larger than 50 µm are present, particle sizing results in underestimation of the average droplet size distribution. Hence the measured relative increase in droplet size is too small. Nevertheless, keeping in mind these restrictions, particle sizing can provide indications on sensitivity to coalescence, although quantitative measurement is difficult.

Emulsions containing less emulsifier and large droplets are expected to be more sensitive than the stable 1 wt% WPI SF and extra stable 1 wt% WPI/NC emulsions. However, the expected large increase in droplets size upon shear (Figure 3.4) was not observed. According to Figure 3.4, OSA-stabilised emulsions were least stable against shear, indicating that these emulsions are not only sensitive to the action of amylase present in saliva but also to shear. Furthermore, Figure 3.4 shows that emulsions containing solid fat are more sensitive to shear than emulsions containing sunflower oil.

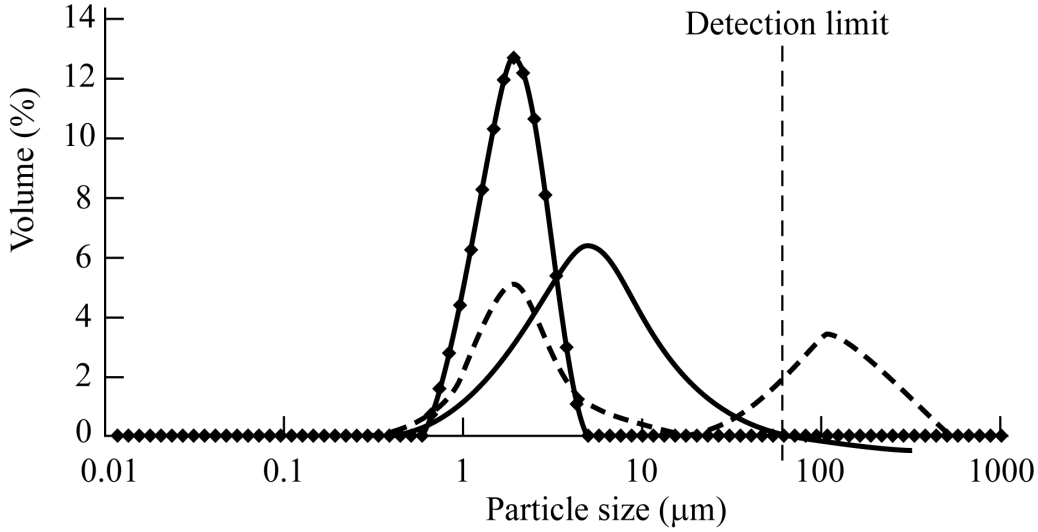

Figure 3.5: Illustration of the systematic error that can occur during particle sizing as a consequence of creaming of large droplets; droplet size distribution of an emulsion before shearing (—◆—) and two distributions after shearing: a) coalescence led to a general increase in the size of all droplets; can be quantified by particle sizing (——) b) Coalescence caused the formation of only a few very large droplets leading to an underestimation of the droplet size distribution (– –)

Coalescence upon oral processing

The same emulsions as used in the sensory panel test were orally processed by various people, making a controlled movement with the tongue and subsequently spitting out the samples. The microscope images of Figure 3.6 show that oral processing a 1 wt% WPI SF emulsion does not result in coalescence of emulsion droplets. Oral processing of

an emulsion stabilized with less emulsifier did result in coalescence. Even larger droplets were observed after oral processing OSA-stabilized emulsions. These droplets were up to two orders of magnitude larger than the original droplet size. The observed effects of oral processing on the different emulsions confirmed our expectation concerning the sensitivity of these emulsions towards coalescence. However, the emulsions containing solid fat did not show the expected instability since they appeared to be more stable than the emulsions with sunflower oil.

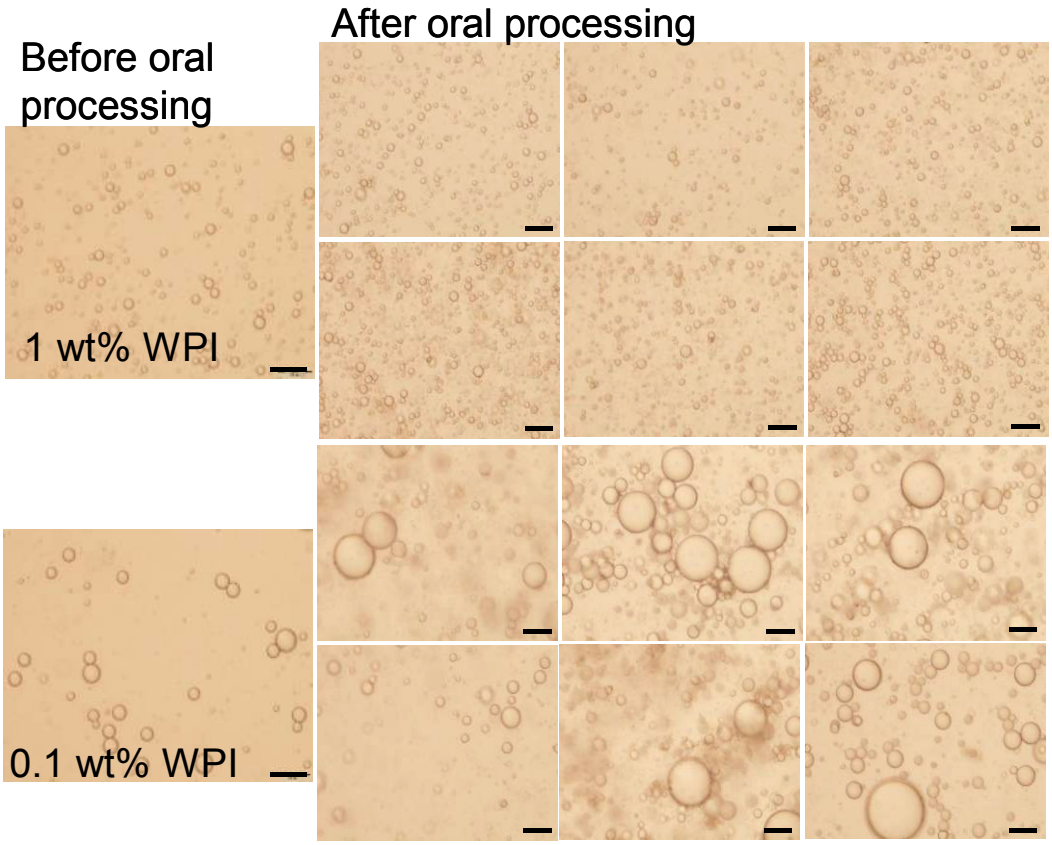

Figure 3.6: Light microscopy images of two emulsions, 1 wt% WPI SF and 0.1 wt% WPI SF containing large droplets: before oral processing and after oral processing by 6 different persons. Scale bar represents 20 μm.

The results obtained with microscopy were verified with light scattering, keeping in mind that particle sizing in these systems is limited to droplets smaller than 50 μm. Figure 3.7 shows the effect of oral processing on emulsion droplet size distribution. Combining the light microscopy and light scattering results indicates that the OSA systems are the least stable systems and the systems containing solid fat are the most stable (see Table 3.4).

The absence of an increase in droplet size upon oral processing of emulsions containing solid fat could relate to the temperature of the mouth. Upon shearing in the OTC an increase in droplet size of those emulsions was found due to occurrence of partial coalescence. However, these OTC experiments were conducted at 22°C instead of body temperature. The probability that partial coalescence occurs increases with the amount of fat crystals in the interfacial layer. The initial temperature of the samples was 22°C and the time to reach 37°C due to in-mouth heating is very short. Also, only 26% of the fat is solid at 22°C. Hence, nearly all crystals would have disappeared at an early stage of oral processing. Most likely the in-mouth conditions were such that the melting time of solid fat is much shorter than the time needed for partial coalescence to occur.

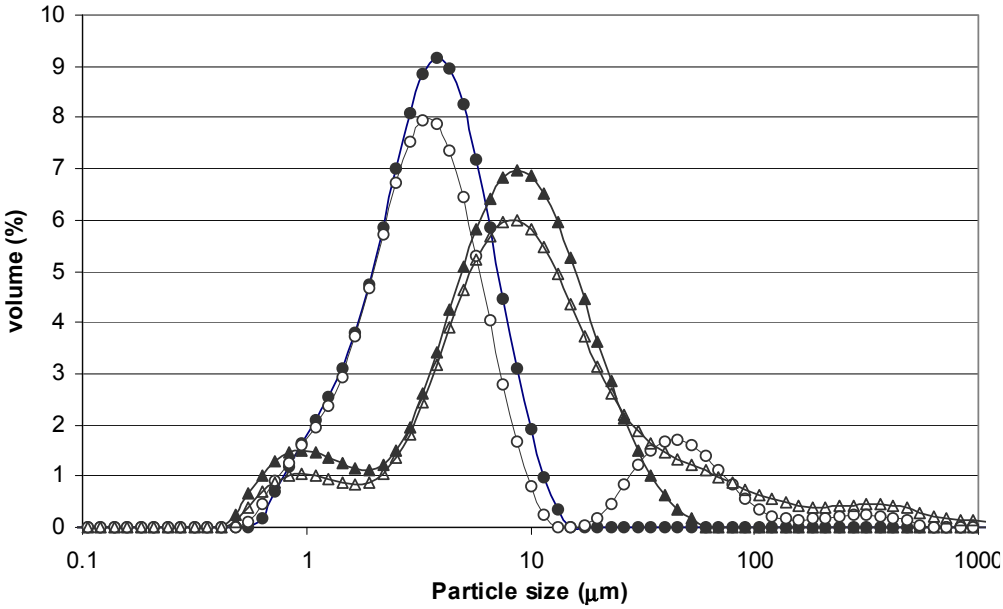

Figure 3.7: Droplet size distribution of emulsions containing initial 'large' droplets determined by light scattering before oral processing: 1 %wt WPI SF (●), 0.1 wt% WPI SF droplets (▲); open symbols after oral processing.

In summary, the results in Table 3.4 largely confirm the expected sensitivity towards coalescences (Table 3.1); emulsions (very) stable (1% WPI & 1% WPI/NC) against coalescence containing sunflower oil are found to be less sensitive to in-mouth coalescence compared to the unstable (0.1% WPI) and very much less sensitive than the amylase unstable emulsion (OSA). However, emulsions containing palm fat are found to be far less sensitive than expected, which is related to the melting of fat in the mouth.

Table 3.4: Overview of the light scattering (LS) and light microscopy (LM) results after oral processing. The numbers in the light scattering column indicate the number of persons out of 6 by which a shift towards larger structures was induced. High numbers in the stability column correlate with a high sensitivity towards coalescence as determined from combining light scattering (LS) with light microscopy (LM).

Emulsion	Shift light scattering	Light Microscopy	Stability from LS and LM
1 wt% WPI /NC SF	6/6	Clusters and some larger droplets	4
1 wt% WPI /NC PF	2/6	Clusters, no enlarged droplets	2
1 wt% WPI SF	6/6	Clusters, no enlarged droplets	3
1 wt% WPI PF	1/6	No clusters, no enlarged droplets	1
0.1 wt% WPI SF	6/6	Clusters and enlarged droplets	5
0.2 wt% WPI PF	1/6	No clusters, no enlarged droplets	1
OSA	6/6	Clusters and very large droplets	6

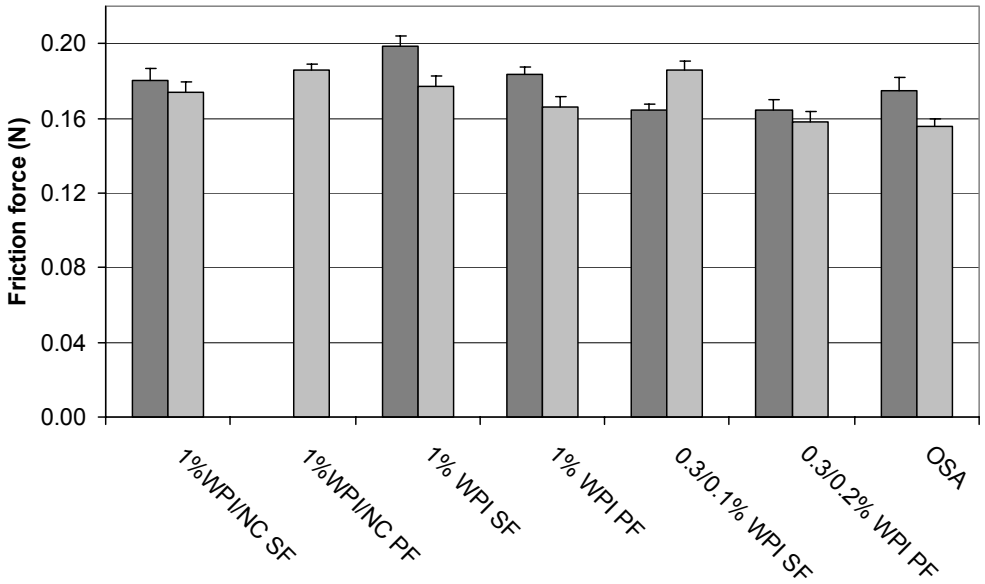

Figure 3.8: Friction force determined by shearing emulsions between pig's tongue and glass. Dark grey columns indicate the small droplets, light grey columns the large droplets. The error bars represent the standard errors.

Effect of coalescence on in-mouth lubrication

Despite the small differences in friction forces between the emulsions, Figure 3.8 suggests that emulsions expected to be unstable against coalescence tend to give the lowest friction forces. Figure 3.8 shows that emulsions with initially large droplets (except 0.3 wt% WPI SF), emulsions containing less emulsifier and emulsions stabilized by OSA gave rise to the lowest friction forces. Comparing the friction data of the emulsions containing sunflower oil with the ones with palm fat indicated that there are different dependencies for the different types of emulsifiers.

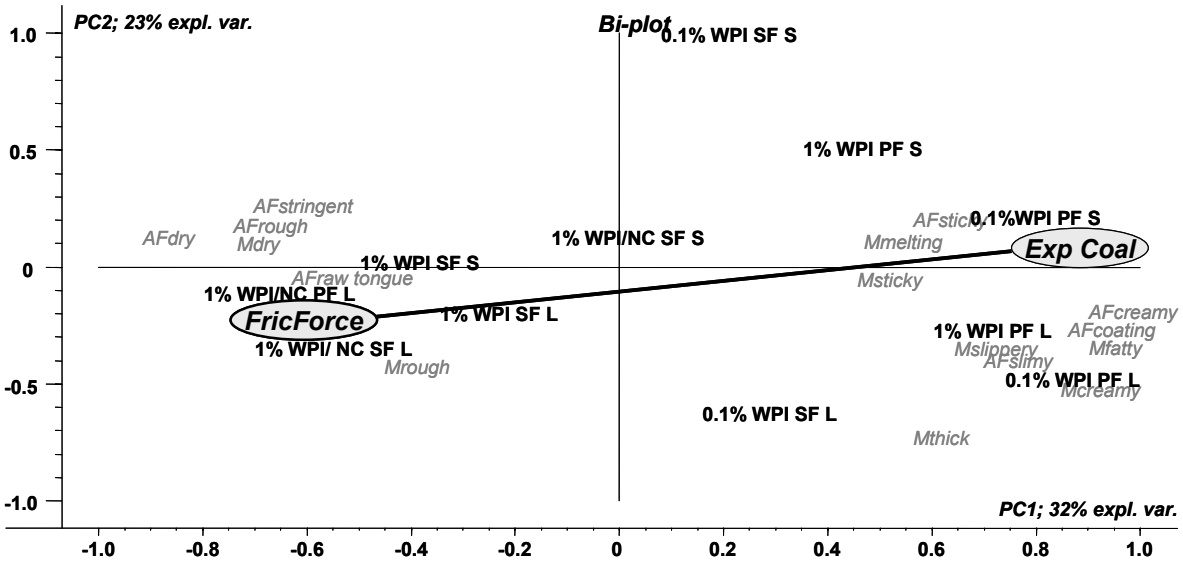

Figure 3.9: Biplot of sensorial evaluation of the different emulsions, the attributes for Mouth-feel (M), After-feel (AF), the expected sensitivity towards coalescence (Exp Coal) and measured friction force (FricForce). OSA emulsions are excluded.

Figure 3.9 shows the results of a principal component analysis (PCA) performed on the friction force data (FricForce), the expected sensitivity to coalescence (Exp Coal) of the different emulsions and the scoring on sensory attributes. The expected sensitivity is plotted since these expectations are, except for palm fat emulsion, confirmed by physico-chemical analyses (see Table 3.4). We already showed that the very unstable OSA emulsions clearly were perceived as more fatty/creamy and less rough than the other emulsions (Table 3.2, figure 3.1). However, the *ex vivo* friction results were obtained in the absence of saliva and thus in the absence of amylase. For this reason hardly any difference in *ex vivo* measured friction was found between the OSA emulsions and the other emulsions whereas *in vivo* OSA was sensed as far lower in friction. Therefore, OSA was omitted from this PCA analysis, in which the measured friction is combined with the sensory scorings. Figure 3.9 shows that along the PC 1 axis a friction force/expected coalescence axis is present. On this axis the emulsions more stable against coalescence and the attributes related to friction, such as after-feel raw tongue, dry, rough, astringent and mouth-feel dry and rough, are positively correlated with friction forces. This means that more stable emulsions are perceived as more rough and thus give rise to a higher friction force upon consumption. Emulsions unstable against coalescence are correlated to the attributes mouth-feel creamy, fatty, mouth filling, slippery and after-feel coating and

satiation. This implies that the perception of fat-related attributes is enhanced by the occurrence of in-mouth coalescence.

3.4 Discussion

Importance of coalescence on sensory perception

Occurrence of coalescence clearly had an effect on emulsion perception. This was particularly clear for OSA-stabilized emulsions. These were not only shown to coalesce rapidly to large droplets (>100 μm) by activity of amylase in the mouth, but also perceived as much more creamy, fatty, thick, slippery and coating, indicating the relation between the occurrence of coalescence in the mouth and perception of these fat-related attributes. The increased perception of fat could definitely not be attributed to the viscosity of the OSA emulsion since all evaluated emulsions exhibit roughly the same viscosity before oral processing (Figure 3.2). The taste intensity and oily taste scorings of OSA emulsions were also higher, suggesting that either both taste and mouth-feel attributes are influenced by coalescence or that it is difficult to judge emulsions on mouth-feel and taste attributes separately.

Differences between the other emulsions with respect to perception of fat-related attributes were small and often not significant. However, by clustering the sensory results and performing ANOVA analyses we could confirm that emulsions that were expected to be more sensitive toward coalescence under shear also gave an increase in the perception of fat-related attributes. For example, droplets stabilized by less emulsifier were perceived as more creamy and fatty than the more stable emulsions with higher emulsifier loads (Tables 3.2 & 3.3). Also, emulsion droplets containing some solid fat instead of just pure liquid oil gave rise to a higher perception of fat (Tables 3.2 & 3.3). However, no significant difference was observed in perception between large and small droplets.

Sensitivity towards in-mouth coalescence

Since less stable emulsions indeed gave rise to an increased fat perception, attempts were made to confirm these expectations on in-mouth instability by performing physico-chemical measurements of droplet sizes. As no single technique is capable of reliably determining very broad size distributions, several techniques were used to gain

insight in the effect of in-mouth processing.

OSA-stabilized emulsion droplets were found to be most sensitive to coalescence. In the spit-out experiment, large droplets were detected; these are probably mainly due to the activity of amylase present in saliva. Also in the (mouth-mimicking) OTC shearing experiments performed in absence of saliva at room temperature, OSA emulsions were most sensitive to coalescence. Other emulsions responded similarly, albeit less outspoken. E.g., emulsion droplets stabilized by less emulsifier showed a large increase in droplet size upon oral processing, but less so after shearing in the OTC. Probably this is due to the inability of light scattering to detect very large droplets, e.g. > 50 μm.

Using palm fat instead of sunflower oil increases the sensitivity towards coalescence albeit in a less pronounced manner, as was verified with the OTC shearing experiment. Rheological experiments did indicate that upon shearing partial coalescence occurred (Figure 3.3), causing the emulsions to be more sensitive towards coalescence in the mouth, since the melting temperature of palm fat is 37°C. However, no increase in droplet size was observed in the spit-out experiments. This is probably due to the fact that solid fat quickly melts in the mouth, well before any partial coalescence can take place.

In contrast to our expectations, no clear difference in sensitivity towards coalescence could be detected between emulsions stabilized by 1 wt% WPI on the one hand and emulsions stabilized by both WPI and Na-caseinate on the other. Furthermore, the effect of droplet size on occurrence of coalescence could not be determined. Possibly, the difference in droplet size between the emulsions with ‘small’ and ‘large’ droplets was not large enough to provoke clear differences in occurrence of coalescence.

To a large extent, the physico-chemical measurements reveal the expected difference in sensitivity towards coalescence and therefore, support the observation that the occurrence of coalescence influences perception. Most importantly, the emulsions found to be most sensitive towards coalescence also gave the highest ratings on fat-related attributes. Apparently, the intensity of creaminess and fattiness perception is determined by the extent to which coalescence occurs.

Contact with the tongue surface (even without shearing) resulted in an increase in the droplet size, suggesting that surface-induced coalescence plays a role as well. The combined effect of contact with the tongue and shearing resulted in a further increase of

the droplet size. Clearly, both shear- and surface-induced coalescence are likely to be of importance for coalescence in the mouth.

Mechanisms by which occurrence of coalescence influences perception

The sensorial evaluation of the different emulsions showed that the taste attributes (taste-intensity, taste-oil and taste-vanilla) followed the same trend as the mouth-feel and after-feel creamy attributes. Furthermore, an increased sensitivity of emulsions towards coalescence resulted in an increased perception of creamy associated taste attributes. This suggests that there is a correlation between the perception of creaminess and fattiness and the perception of taste, which could be attributed to an enhanced aroma release caused by the occurrence of coalescence. The perception of fat is probably related to a combination of aroma release and lubrication.

In this work we focussed more on unravelling the physico-chemical mechanisms by which occurrence of coalescence could influence perception. In-mouth coalescence of emulsion droplets lowers the perceived oral friction, which results in an enhanced perception of fat. This can be concluded from Figure 3.9, a PCA-plot in which the friction data are combined with the expected sensitivity towards coalescence and the sensory perception of the different emulsions. Figure 3.9 shows that indeed friction forces and expected coalescence had an opposite position on the PCA 1 axis. There appears to be a correlation between friction-related attributes such as after-feel raw tongue, dry, astringent, rough and mouth-feel rough and friction forces. The expected sensitivity towards coalescence seems to be positively correlated with mouth-feel creamy and fatty, and after-feel coating. Thus, friction forces sensed in the mouth play a role in perception of fat-related attributes.

We cannot conclude from the data which mechanism is responsible for lowering the friction. Our current hypothesis is that coalescence influences the lubrication of tongue tissue either by forming a fatty coating on the tongue surface due to spreading of emulsion droplets on the tongue, or because of formation of larger oil droplets, which upon shearing between the palate and tongue give rise to an increased hydrostatic pressure, that keeps tongue and palate apart thus lowering the friction. Further research is being carried out to further clarify these points.

3.5 Conclusion

Emulsions with a higher sensitivity towards coalescence were also perceived as most fatty and creamy. This gives strong indications that in-mouth coalescence of emulsion droplets enhances the perception of fattiness and creaminess. The results indicated that both aroma release and mouth-feel are affected by the occurrence of coalescence and that both factors had an effect on perception of creaminess and fattiness. Friction force measurements performed under mouth-mimicking conditions showed that emulsions, which were more sensitive towards coalescence, gave rise to a lower friction force. The results of the sensory panel confirmed that the *ex-vivo* measured lowering of the friction forces was also perceived sensorially.

Occurrence of coalescence in the mouth is depending 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. Emulsions either stabilized by less emulsifier, stabilized by OSA or, to lesser extent, containing solid fat, were most sensitive towards coalescence. In the mouth both shear-induced coalescence and surface-induced coalescence were found to play a role.

This work indicates that, in principle, low fat products with a full fat sensation can be produced by varying the sensitivity towards coalescence. Further research on the mechanisms involved in fat perception in relation to occurrence of coalescence is ongoing.

Acknowledgements

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Appendix

Table A1: Descriptions given by the QDA panel

Odour attributes		**After-taste attributes**	
Ointensity	Intensity of the odour	ATsweet	Sweet aftertaste
Ovanilla	Vanilla odour; smell like babyfood (milk)	ATbitter	Bitter; taste like aspirin; sparkling feeling in the rear of the mouth
Ochalk	Odour of chalk	ATmetal like	Metal; ironlike; oxidized
		ATduration	Duration of taste perception in the mouth after swallowing
Taste attributes		**After-feel attributes**	
Tintensity	Intensity of the taste; taste-explosion	AFastringent	Astringent; contracting after-feel
Tvanilla	Taste of vanilla	AFcreamy	Velvety; warm; soft
Tsweet	Sweet taste	AFdry	Saliva absorbing; dry tongue
Tsour	Sour taste; like citrus fruit	AFrough	Rough feeling on the teeth
Tsalty	Slightly salt taste, like liquorice, seawater	Afraw tongue	Raw feeling; sandpaper or cat's tongue
Toil	Taste of salad oil	AFmealy	Fine particles, floury, powdery
Tfakecream (Tcream)	Taste of imitation cream	AFslimy	Slippery like porridge
Tglue	Taste like potato- or maize starch	AFcoating	Fatty coating on tongue, lips or cheek
Mouth-feel attributes		**After-feel attributes**	
Mthick		AFsticky	Syrupy; stickily
Mcreamy	Velvety; warm; soft	AFsatiation	Hunger alleviation; filling; satisfactory
Mfatty	Oil-like; fatty layer in the mouth	AFtingling	Pungent; tingling feeling on lips and tongue
Mslippery	Slippery feeling		
Msticky	Sticky, tacky		
Mrough	Rough feeling on the teeth and/or tongue		
Mdry	Dry feeling in the mouth; saliva is absorbed		
Mmelting	Dissolves in the oral cavity; structure vanishes		
Mmouthfilling	Feeling that whole the mouth is filled up		

4

Tribology of o/w emulsions under mouth-like conditions: determinants of friction

Abstract

Fat-perception is thought to be related to a complex interplay between fat-associated flavour release and mouth-feel. Friction sensed between the tongue and the palate seems to play a prominent role: in previous work we have shown that emulsions that are more sensitive towards coalescence give rise to a lowering of the orally perceived and experimentally measured friction, and, probably as a consequence, to an enhanced fat-perception.

Here, we study in detail the factors determining friction of protein-stabilised emulsions using a novel mouth-mimicking tribometer and model surfaces consisting of PDMS modified in various ways (hydrophobicity, deformability, roughness). We show that, unlike in many technological applications where lubrication is essentially hydrodynamic, for physiologically relevant loads, the modified PDMS is boundary and/or mixed lubricated, which is like in-mouth lubrication.

We find that an increased sensitivity of the emulsions towards coalescence results in a lower friction, confirming previous results obtained with pig's tongue. Surface-induced coalescence (or spreading of emulsion droplets) seems to be very important in this, surface hydrophobicity being the dominant trigger. Viscosity of the dispersed phase does not have a strong influence on both the measured friction and the oral perceived friction. We do find a strong influence of the presence of bulk proteins and saliva on friction. Finally, hardly any dependence of measured friction on fat content of the emulsion was observed, indicating that only a small amount of fat is needed to alter the friction.

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4.1 Introduction

Designing food emulsions that contain less fat, but nevertheless give a full-fat sensation is of great commercial and public interest. Fat perception is considered to be related to both fat-associated flavour release and mouth-feel. Mouth-feel of food is often linked to rheological in-mouth behaviour. However, various authors suggested that rheological behaviour alone can not explain mouth-feel, but that a relation between in-mouth friction and fat-related mouth-feel can [1-5]. We demonstrated an inverse relation between perception of fat and in-mouth friction sensed between tongue and palate (Chapter 3). Also, we showed that food oil-in-water (o/w) emulsions, which are sensitive towards coalescence give rise to a lower orally perceived and measured friction, and, probably as a consequence, have an enhanced fat perception. Although knowledge on the relation between friction and fat perception is still rather limited, it is clear that understanding the tribology of food emulsions under mouth-like conditions would be an important step towards understanding fat perception. However, at present, even a basic understanding of emulsion lubrication in general [6], and more specific, under mouth-like conditions is lacking [7].

In contrast, friction, lubrication and wear (or in short, tribology) have been extensively studied, for example, in the context of metal processing and other technologies. In tribology three regimes of lubrication are typically distinguished, which are often identified by analyzing the shape of the so-called Stribeck curve (friction coefficient plotted against the sliding speed (or film thickness), illustrated in Figure 4.1). For most technological applications, the focus lies on how to minimize wear and optimize lubrication of the surfaces to reduce energy consumption. Therefore, attention is usually restricted to the *hydrodynamic lubrication* regime. In this regime the opposing surfaces are completely separated upon sliding due to build-up of a hydrodynamic pressure as a function of speed. This means that the ability to form a hydrodynamic film depends mainly on the viscosity of the lubricant and surface characteristics are of minor importance.

On the other hand, the tongue has a rough surface due to the presence of papillae (height several hundreds of micrometers, see Chapter 2), which allows food handling for

mastication purposes. Combined with the low speed of sliding [8] and low contact pressures, it is clear that surface characteristics are crucially important for in-mouth lubrication, and that in-mouth lubrication is not in the hydrodynamic regime, but rather in the so-called *boundary* regime (where friction depends on the characteristics of the surfaces including the thin adsorbed boundary layer), or in the *mixed* regime, which forms the transition between the boundary and the hydrodynamic regime.

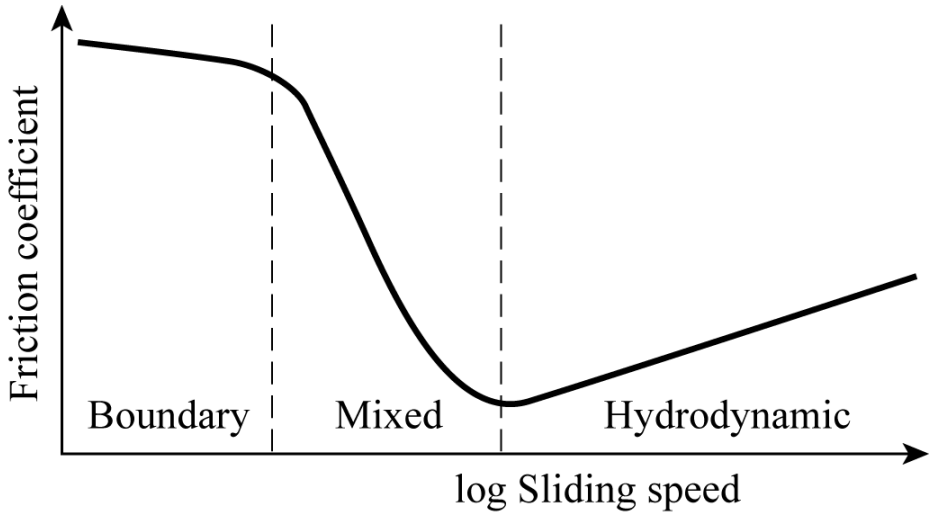

Figure 4.1: Stribeck curve with the three lubrication regimes.

In order to understand how lubrication by food oil-in-water (o/w) emulsions can influence in-mouth friction and thus fat perception, we recently developed a mouth-mimicking tribometer, the Optical Tribological Configuration (OTC, see Chapter 2). In contrast to the circular movement employed in traditional tribometers, in this tribometer two surfaces slide over one another in a relative parallel movement. The parallel sliding movement has the advantage of being more similar to the in-mouth situation of the tongue moving parallel to the palate than the circular rolling movements. Disadvantage of the parallel movement is that the ability to measure at very high speed is limited. Furthermore, the OTC allows the surfaces to move over a distance comparable to the tongue-palate situation. Also, low loads of tenths of Newtons can be applied resulting in a contact pressure comparable to the in-mouth situation. At these low loads, wear phenomena are found to be negligible. Another feature of this tribometer is that we are not restricted to synthetic surfaces, but can also use biological materials. For example, we have recently used the OTC with pig's tongue to mimic in-mouth lubrication (Chapter 2

and 3). While there are clear reasons for studying tribology with real biological surfaces such as pig's tongue, there are clear disadvantages too: limited availability, variability between the tongue samples and especially the inability to alter the surface characteristics to test hypotheses and dependencies.

For these reasons, in this study, we use modified Poly-DiMethylSiloxane (PDMS) to complement our studies using pig's tongue. The use of PDMS allows us to systematically alter a single surface characteristic, such as the roughness, deformability and hydrophobicity, without changing the others. Velocities reached in the OTC are similar to typical in-mouth velocities of food as was estimated from data on shear rates in the mouth [8]. We exclusively use rough surfaces since the aim is to avoid the less relevant hydrodynamic regime of lubrication and instead focus on the boundary and/or mixed regime. Also, rough surfaces give rise to complex flow behaviour between the asperities, much like in the mouth (Chapter 2). The tongue surface is not only rough, it also is notably hydrophobic, and highly deformable (Chapter 2). Therefore, using our PDMS model surfaces we vary not only roughness, but also hydrophobicity and deformability. Presumably, these factors will influence the formation of lubricating layers by emulsion components, which can influence friction, for example by altering adhesion between the surfaces.

Friction is not only determined by surface characteristics, but of course also by the lubricating fluid itself. In our previous studies we have found a clear influence of the emulsions' sensitivity towards coalescence. This dependence will also be addressed in this study, now using the controlled PDMS surfaces. Furthermore, since it has been shown that the oil viscosity has a pronounced effect [9] when droplets are not stabilised by a surfactant, we will address the influence of oil viscosity on friction and relate this to the sensorially perceived friction of the emulsions.

Finally, for in-mouth lubrication it is impossible to ignore the role of saliva [10-12]. Most likely, saliva proteins (e.g. the mucin glyco-proteins), adhere to surfaces and influence friction in the boundary and mixed regime by changing adhesion between the surfaces. When considering protein-stabilised emulsions, the same considerations hold for the proteins in the aqueous phase. Therefore, we included a study on both the role of saliva, and of bulk-phase proteins.

4.2 Material and methods

4.2.1 Emulsion preparation

O/W emulsions containing 40 wt% oil were prepared according to the procedure described in Chapter 3. Vanilla, sucrose and salt were only added to emulsions that were sensorially tested. The emulsions were stabilised by either 1 wt% Whey Protein Isolate (WPI; BiPro, Davisco, USA) or 0.3 wt% WPI. Sunflower oil (SF; fully winterized sunflower oil, η= 60mPa.s, gift by Cargill Amsterdam, The Netherlands) was used as the oil phase. The emulsions were diluted with the continuous phase to a 20, 10, 5 and 1 wt% SF oil emulsion. The 1 wt% WPI stabilised emulsions were previously shown to be stable against coalescence under shear in the tribometer and in the mouth, while the 0.3 wt% WPI stabilised emulsion was shown to be unstable (Chapter 3). Furthermore, emulsions stabilised by 1 wt% WPI were formulated with MCT oil (Miglyol 812N, Condea Chemie, Witten, Germany, η= 30 mPa.s), Olive oil (Olivae oleum virginale Fagron, Nieuwerkerk a/d Ijssel, The Netherlands, η= 81 mPa.s) and Castor oil (Ricini oleum virginale, Fagron, Nieuwekerk a/d Ijssel, The Netherlands, η= 980 mPa.s).

The average droplet size of the emulsions was 1.1 μm, D[3,2], and 2.4 μm D[4,3]. The viscosity of the emulsions varied between 4.9 mPa.s (at 90 s^{-1}) for 40 wt% oil emulsions to 2.1 mPa.s for 20 wt%, 1.4 mPa.s for 10 wt%, 1.3 mPa.s for 5 wt% and 1.1 mPa.s for 1 wt% oil emulsions.

4.2.2 Tribopairs

A Poly-DiMethylSiloxane (PDMS) pin was manufactured according to the method described in Chapter 2 and by Lee and co-workers [13]. A commercial silicone elastomer kit (SYLGARD 184 DOW Corning, Midland USA) containing a base and a cross linker was used. The cross linker was mixed with the base in a ratio 1:10 for "hard" pins (0.64 MPa) and in a 1:20 ratio for "soft" pins (0.16 MPa). The elastomer was cured in 96-wells plates, which were sandblasted at two different pressures and served as moulds. The resulting pins had an average asperity height of around 4.5 μm (roughness high, RH) and 2 μm (roughness low, RL). The radius of the spherical tip is 3 mm. The water contact angle against air for the "hard" pins was 108° and for the "soft" pins 90°. The pins were

hydrophilised following the method of Lee et al [13] by oxygen-plasma-treatment for 2 minutes in a plasma-cleaner (Harrick PDC-32 G, Anadis instruments, Malden, The Netherlands). Oxidation of the surface altered the chemical characteristics to such an extent that it became hydrophilic. Note that the minor alterations in the chemical composition of the surface due to oxidation might also give rise to minor changes in other surface properties. The water contact angle on these oxidised PDMS surfaces was less than 5°. The pin was slid against a microscope glass cover slip (R_a = 2 nm, water contact angle 65°).

4.2.3 Tribological study

Tribology measurements were performed with the Optical Tribological Configuration (OTC), which is able to measure forces down to 8 mN. Roughly the same method was used as described in Chapter 2. In short, an amount of 150 µl emulsion was sheared in the OTC between PDMS/glass under a load (F_z) of 0.5 N. During one experiment the lower glass plate oscillated ten cycles over a distance of 16 mm against the upper PDMS pin. Simultaneously, the friction force (F_x) was measured and the average friction force was calculated over the span of the movement where the speed of shearing was constant. The sliding speed varied from 0.01 m/s until 0.08 m/s. A speed of 0.01 m/s was chosen as the lower limit since a lower speed implies an increase in duration of the experiment resulting in dehydration of the sample.

Each experiment was carried out 3 times using new PDMS pins and fresh emulsions for every experiment. The PDMS pins were placed into the OTC measuring probe in such a way that the pins were spherical-shaped.

4.2.4 Saliva

To study the influence of saliva, the PDMS surfaces were coated with unstimulated saliva by covering the surface with an excess of saliva during 2 minutes. Unstimulated saliva was used since this contains the highest concentration of salivary proteins. It was found by Silletti *et al*. [14] to give saliva-induced flocculation of emulsion droplets. Saliva was donated by 5 subjects (following the protocol of Silletti and co-workers [14]) who refrained from eating for 2 hours before donation. The subjects thoroughly rinsed their

mouth before donating. The saliva was kept on ice during donation and then centrifuged to remove cells. Saliva was frozen in liquid nitrogen, stored at -80°C and used within 6 weeks after donation.

4.2.5 Sensory Study

The emulsions were sensorially evaluated following the same procedure as described earlier (Chapter 3) by a sensory trained panel according to the principles of Quantitative Descriptive Analysis [15]. In short, a panel of 8 female panelists generated in separate sessions descriptive attributes of which 38 were used to profile the 4 different emulsions (Chapter 3). The panellists were seated in climate-controlled sensory booths and judged the set of emulsions in a semi-monadically assessment procedure in triplicate on visual analogue scales. The acquisition was done by computer using FIZZ software (Biosystemes 2006, v2.20 A 2006 , Couternon, France). The emulsions were evaluated on 4 odour (O), 10 taste (T), 9 mouth-feel (M), 4 after-taste (AT) and 11 after-feel (AF) attributes.

4.3 Results

Before discussing the data on lubrication by protein-stabilized o/w emulsions, we first consider lubrication by oil and water separately, for hydrophobic and hydrophilic PDMS, and for PDMS surfaces differing in roughness. All Figures presented in this section have error bars (representing the standard errors). In cases where these are invisible, they are smaller than the symbol size.

4.3.1 PDMS lubrication

Oil wets hydrophobic surfaces much better than hydrophilic surfaces, hence oil may be expected to be a better lubricant for hydrophobic than for hydrophilic surfaces. Indeed, Figure 4.2 shows that low viscosity MCT oil lubricates hydrophobic PDMS (Pho) much better than it lubricates hydrophilic PDMS (Phi). Increasing the viscosity of the oil (SF, Castor) further reduces the measured friction on hydrophobic PDMS. Note however, that this also decreases the difference with lubrication of hydrophilic PDMS.

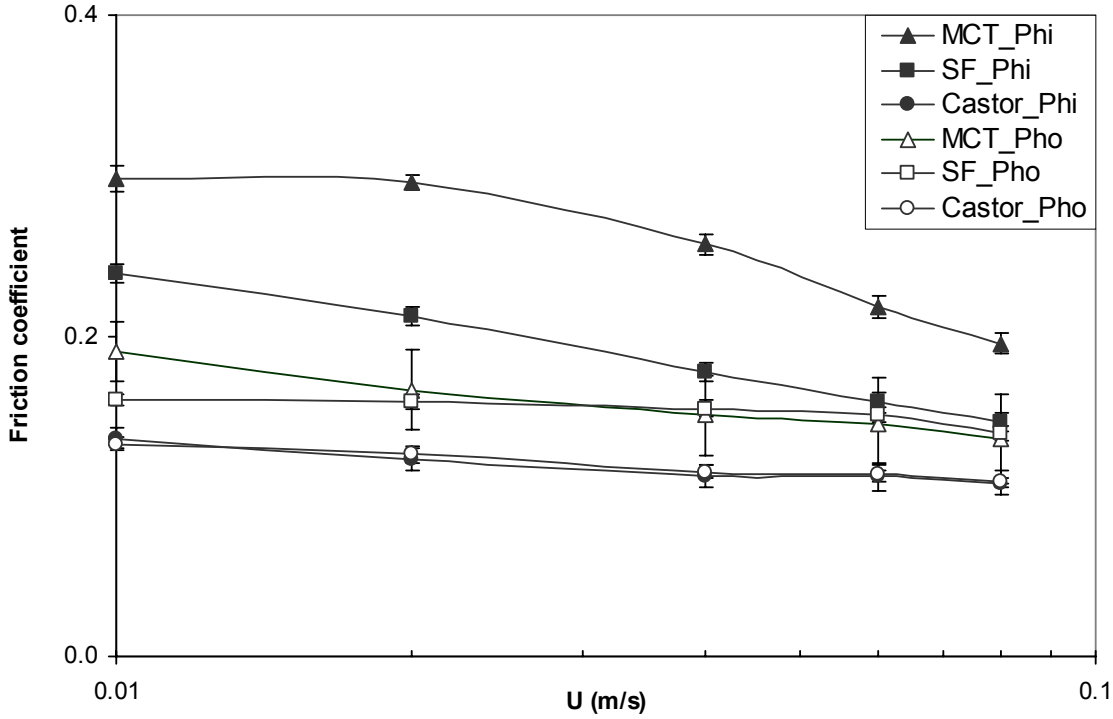

Figure 4.2: Friction coefficient as a function of speed (U) with oil varying in viscosity sheared between hydrophobic (open symbols) and hydrophilic (closed symbols) hard PDMS with roughness low (RL); MCT oil —▲—, Sunflower oil —■— and Castor oil —●—.

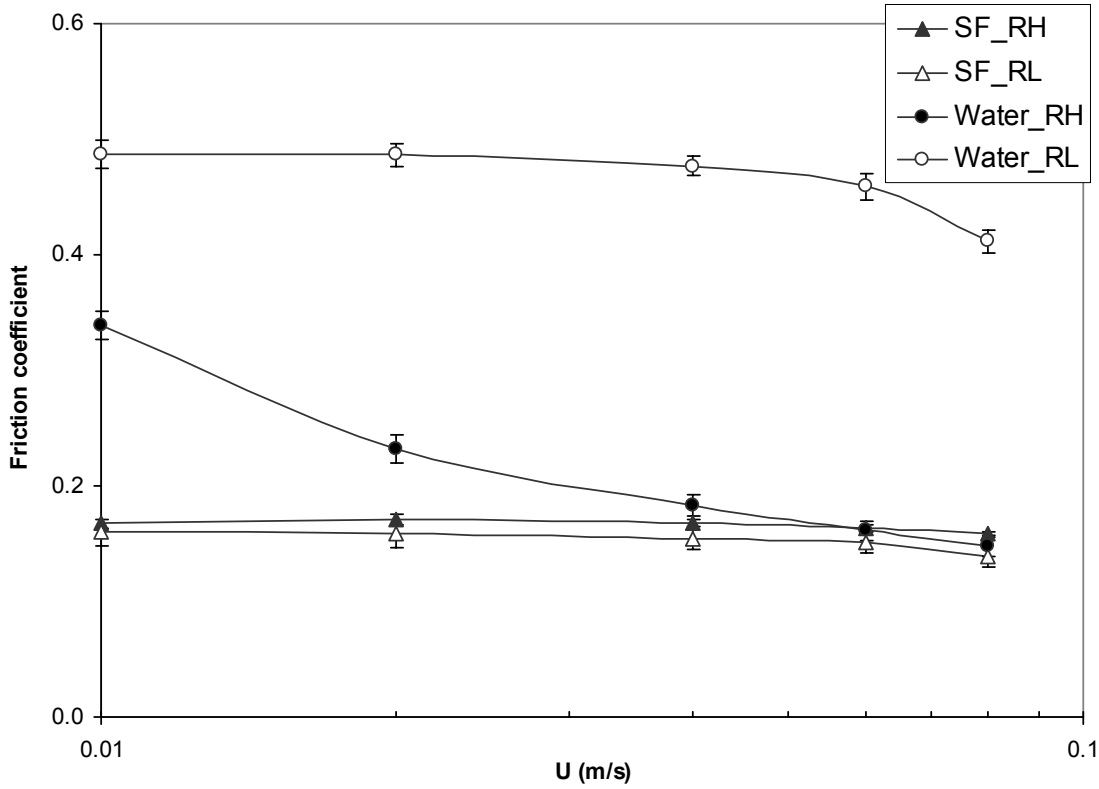

Figure 4.3: Friction coefficient as a function of speed (U) with hard PDMS (hydrophobic) varying in roughness high (RH; closed symbols) and low (RL; open symbols) with the lubricants sunflower oil —▲— and water —●—.

As explained in the introduction, in the hydrodynamic and, to lesser extent, in the mixed regime, friction is mainly determined by the viscosity of the lubricant, rather than the surface properties (Figure 4.1). For all oils, even for the highly viscous Castor oil, we observe a gradual decrease in the friction, as function of speed. Characteristic for the hydrodynamic regime is an increase in friction as function of speed (see Figure 4.1). No increase in friction is observed in Figure 4.2 indicating that for the rough PDMS surfaces used here we have boundary and/or mixed lubrication (see Figure 4.1). The fact that for the most viscous oil (Castor) we find very little difference between lubrication of hydrophilic and hydrophobic PDMS may indicate that for this oil we do indeed approach the hydrodynamic regime. On the other hand, for the least viscous oil (MCT), there is a clear surface effect and hence, in this case we are in the boundary/mixed regime over the speed range tested here.

Besides surface hydrophobicity and viscosity of the lubricant, surface roughness is an important factor in lubrication. Indeed, comparing smooth PDMS (see Chapter 2) with the rough PDMS used here we see a strong influence of roughness on lubrication. Figure 4.3 shows a clear difference in friction coefficient between hydrophobic surfaces with a low surface roughness (PDMS-RL) and high surface roughness (PDMS-RH) when water is the lubricant, but not when sunflower oil is the lubricant. Water is a better lubricant for PDMS surfaces with high surface roughness, than for surfaces with low surface roughness. On the other hand, when sunflower oil is the lubricant, roughness does not seem to influence friction very strongly. Apparently, for this case, the higher viscosity, together with the ability to wet the hydrophobic surface, implies that we are further away from the boundary regime where surface properties are dominant. However, the (slight) decrease in friction as function of speed indicates that also in this case the lubrication of the surfaces is boundary/mixed.

For none of the measured combinations do we see strongly *increasing* friction coefficients as function of sliding speed. Increasing friction as function of speed is characteristic for the hydrodynamic regime. Even though we cannot access a wide enough range of loads and speeds to cover the entire Stribeck curve, we can conclude that under the conditions that we use, we are certainly *not* in the hydrodynamic regime. Since surface properties not always dominate lubrication and viscosity of the lubricants sometimes does

play a role, we are not always in the boundary regime either. In short, under the conditions we apply in the OTC and using modified PDMS surfaces, the regime of lubrication is boundary and/or mixed, but certainly not hydrodynamic. Next we consider lubrication of PDMS by food emulsions.

4.3.2 Surface properties

We investigated (using PDMS) the influence of surface hydrophobicity and deformability on emulsion lubrication, for emulsions of fixed composition. First, however, we briefly compare emulsion lubrication with lubrication by the oil and water separately. One might expect that, as long as the emulsion droplets do not stick to the PDMS and remain stable, emulsion lubrication should be similar to water lubrication considering the fact that the viscosity of the continuous phase is almost equal to water. However, Figure 4.4 clearly shows that even the unstable emulsion is always a worse lubricant than either oil or water. In section 4.3.4, we will return to this finding when discussing the role of emulsions stabilizers (proteins) and the role of saliva. Despite the observation that the emulsion does not resemble either water or oil in terms of friction, it lubricates hydrophobic surfaces better than hydrophilic ones. This could be explained by the fact that oil tends to spread on hydrophobic surfaces, which happens when emulsion droplets coalesce on the surface. On hydrophilic surfaces, this tendency is much less strong, even in the presence of proteins.

As for the deformability, Figure 4.5 shows that the difference between soft and hard PDMS lubrication lies mainly in the shape of the (partial) Stribeck curve. For soft PDMS the friction is almost constant as function of the speeds, and only starts to decrease at the highest sliding speed. On the other hand, for hard PDMS, the friction is gradually decreasing with the sliding speed, indicative for mixed lubrication. In other words, an increase in deformability of the surfaces leads to an extended boundary regime, since the adhesion between soft surfaces is higher than between hard surfaces.

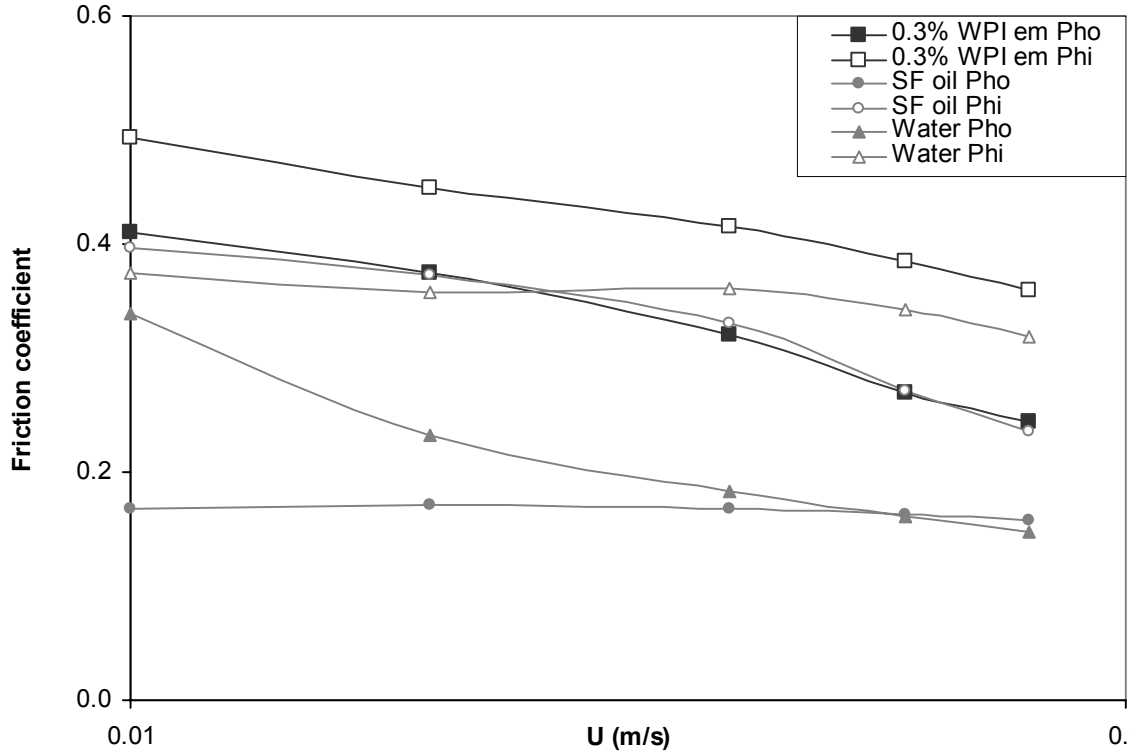

Figure 4.4: Friction coefficient as function of speed (U) of 0.3 wt% WPI 40 wt% SF emulsion —■— sheared between hydrophobic hard PDMS (closed symbol) and hydrophilic hard PDMS (open symbol) in comparison to SF oil —●— and water —▲— . Roughness is RH.

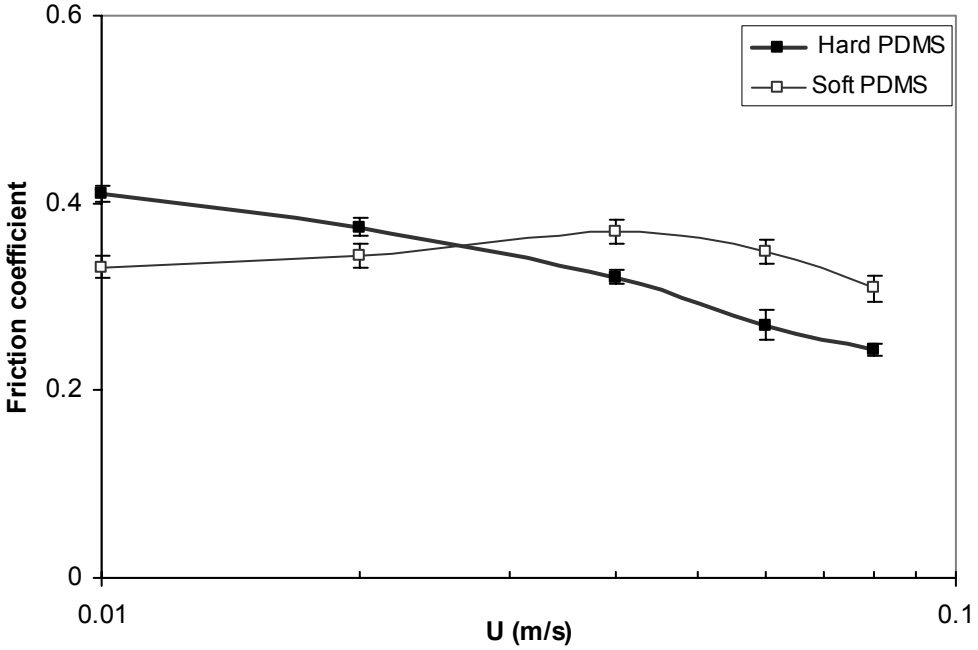

Figure 4.5: Friction coefficient as a function of speed (U) with PDMS (hydrophobic, RH) varying in deformability, hard PDMS (closed symbols), soft PDMS (open symbols), with an emulsion (0.3 wt% WPI, 40 wt% SF) acting as a lubricant.

4.3.3 Emulsion characteristics

So far we have only considered emulsions of a fixed composition and considered the influence of surface properties on friction. Next, we will also vary emulsion properties and see how these influence lubrication. Here, we study the dependence of friction on emulsion stability, fat content, viscosity of the dispersed phase using our model PDMS surfaces. In addition the effect of viscosity of the dispersed phase on oral perceived friction is determined. First, consider the role of emulsion (un)stability in lubrication. We expect that surface-induced coalescence, possibly facilitated by shear-induced coalescence (see insert Figure 4.7), is capable of lowering the friction. By surface-induced coalescence we here mean the process in which oil droplets, either stabilised or not, spread on a solid surface. More surface-induced coalescence will primarily occur on hydrophobic surfaces and with unstable emulsions. Indeed, we see in Figure 4.6 that the gradual decrease in friction as function of speed (typically for mixed lubrication) for the unstable (0.3% WPI emulsion) is steeper than for the more stable, 1% WPI emulsion on hydrophobic PDMS. Hence, the unstable emulsion gives rise to lower friction than the stable

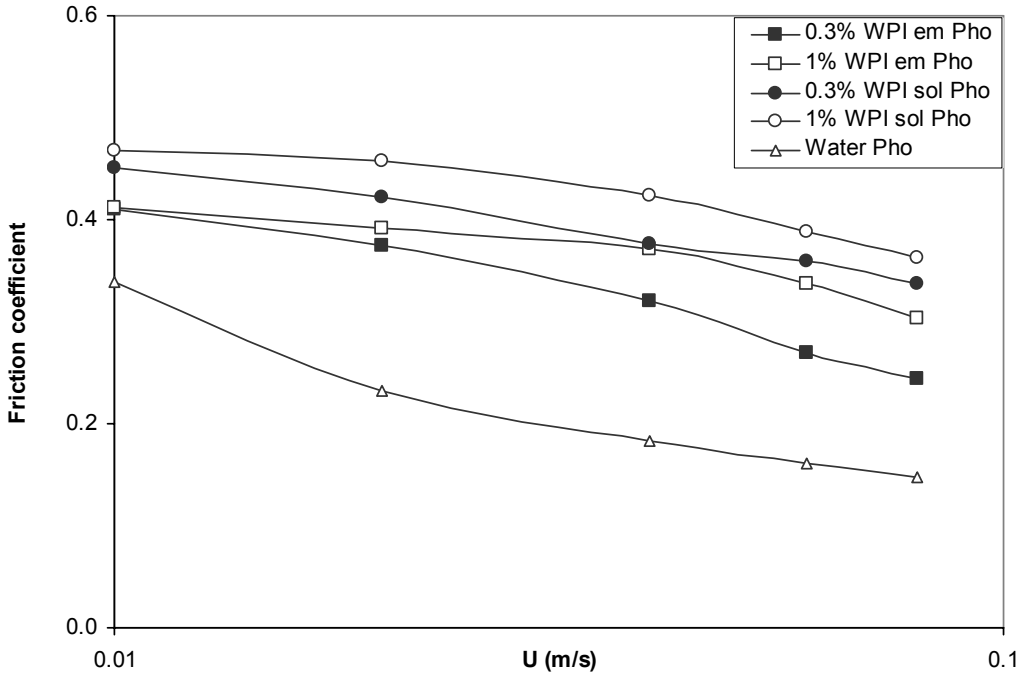

Figure 4.6: Influence sensitivity to coalescence on friction; friction coefficient as function of speed (U) with emulsions, 0.3 wt% WPI 40 wt% SF —■— and 1 wt% WPI 40 wt% SF —□—, water —△— and protein solutions, 0.3 wt% WPI solution —●— and 1 wt% WPI solution —○—, sheared between hydrophobic hard PDMS, roughness is RH.

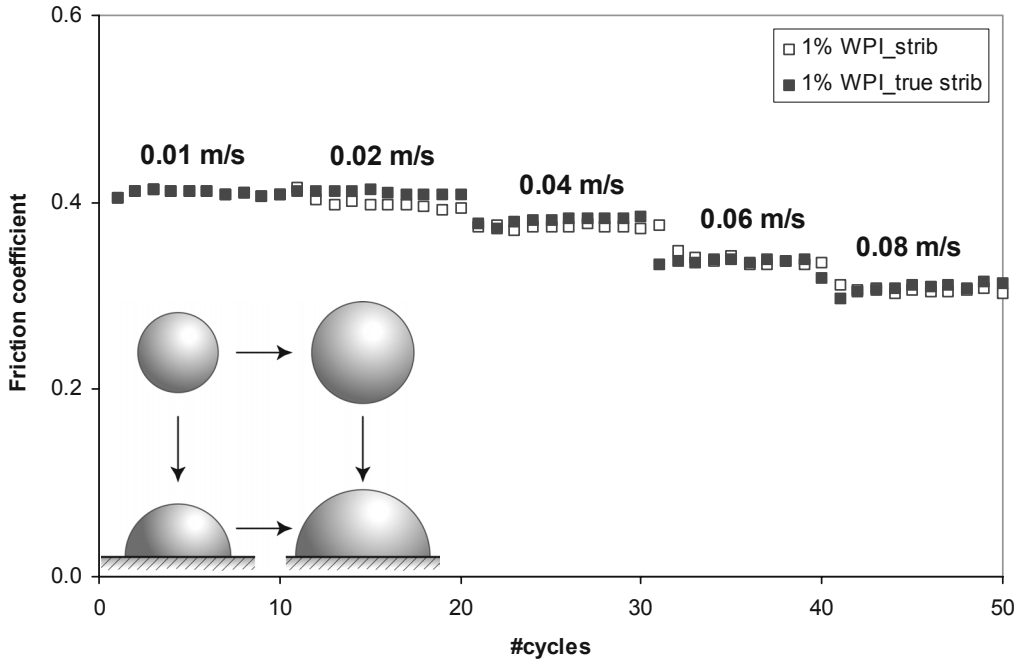

Figure 4.7: Friction coefficient measured for every cycle at speeds varying from 0.01 until 0.08 m/s between glass and hard hydrophobic PDMS-RH with 1 wt% WPI 40 wt% SF —□— as a lubricant in comparison to the friction coefficient measured for the different speeds separately, 1 wt% WPI 40 wt% SF —■— (true Stribeck curve). The insert shows how shear-induced coalescence can facilitate surface-induced coalescence.

emulsion. Figure 4.6, also shows that protein solutions are worse lubricants than either an emulsion or water. The role of protein in lubrication will be discussed in detail in further sections. In brief, unstable emulsions are more effective in lowering of the friction than stable emulsions.

The question is, can this difference in emulsion stability be explained in terms of sensitivity to shear-induced coalescence, or is it mainly due to a stronger tendency to surface-induced coalescence? To determine whether the stable 1% WPI emulsion was sensitive to shear we checked the influence of shear history on the friction, and sheared the stable emulsion in different ways: either using step-wise increases in speed on a single emulsion that remained in the OTC, or by using fresh emulsion for each new speed. We expect that if shear-induced coalescence would occur at a certain speed, the presence of the enlarged droplets (which are more sensitive towards further coalescence; shear and/or surface-induced) would affect the Stribeck curve at the following higher speeds. As is evident from Figure 4.7, there seems to be no effect whatsoever of shear history, indicating that hardly any shear-induced coalescence has occurred and that lowering of the

friction with the speed was mainly due to entering deeper into the mixed regime. Thus unstable emulsions indeed give rise to a lower friction than stable emulsions on hydrophobic PDMS. The distinction whether this difference is due to a difference in sensitivity to shear could not be made. The viscosities of both emulsions are very similar, so the differences that we observe must have their origin in properties of the lubricating layer, indicating that surface-induced coalescence is likely to play a prominent role.

Fat content

Reduction in friction by emulsions is expected to be mainly determined by the formation of an oil film, as a result of surface-induced coalescence. Therefore, we expect a critical amount of fat to be necessary to form oil patches, which significantly reduce the friction. Surface roughness probably affects how easily such layers are formed and, hence, was also taken into account as a variable.

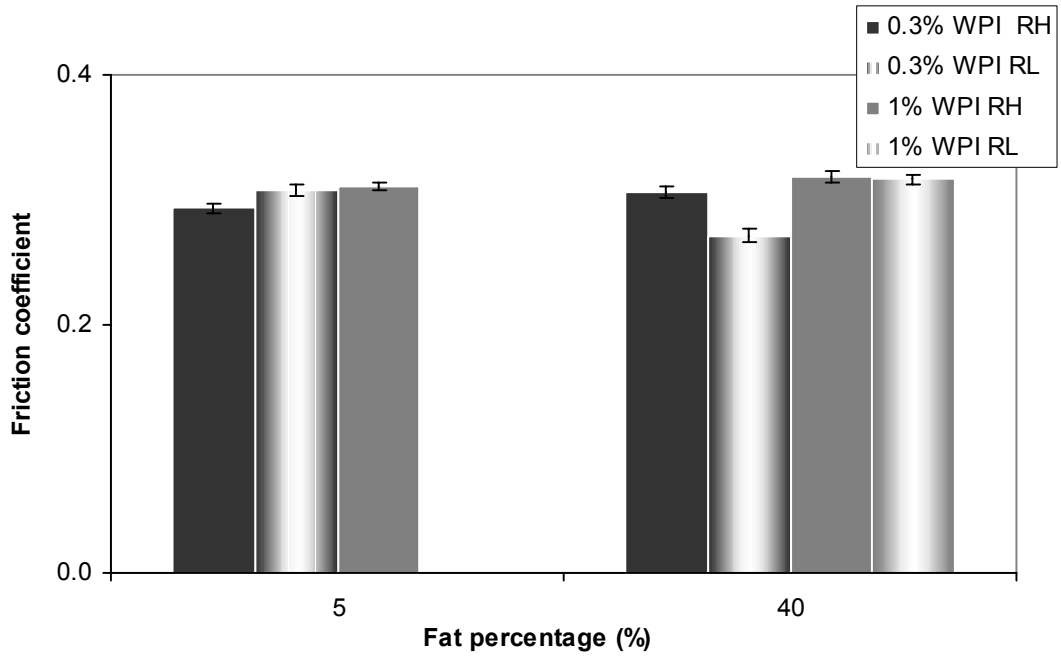

Figure 4.8: Friction coefficient as function of fat percentage of two different emulsions 0.3 wt% WPI emulsion (black) and 1 wt% WPI emulsions (grey) sheared between hydrophobic hard PDMS RH (filled) and RL (duo tone) sheared 0.08 m/s.

Figure 4.8 shows that, except for the 0.3 wt% WPI 40wt % SF emulsion, there is hardly any dependence on surface roughness at a sliding speed of 0.08 mm/s. Possibly, this is because of the relatively small difference in surface roughness (mean asperity height 2 μm vs. 4.5 μm). Indeed, we did find a substantial decrease in friction when

comparing the 4.5 μm PDMS surface roughness with a very smooth (2 nm, see Chapter 2) PDMS surface roughness (data not shown). The most remarkable effect is, however, that there is hardly any difference in friction between a 5 wt% and a 40 wt% fat emulsion. Apparently, only a small amount of oil is needed to form a lubricating layer.

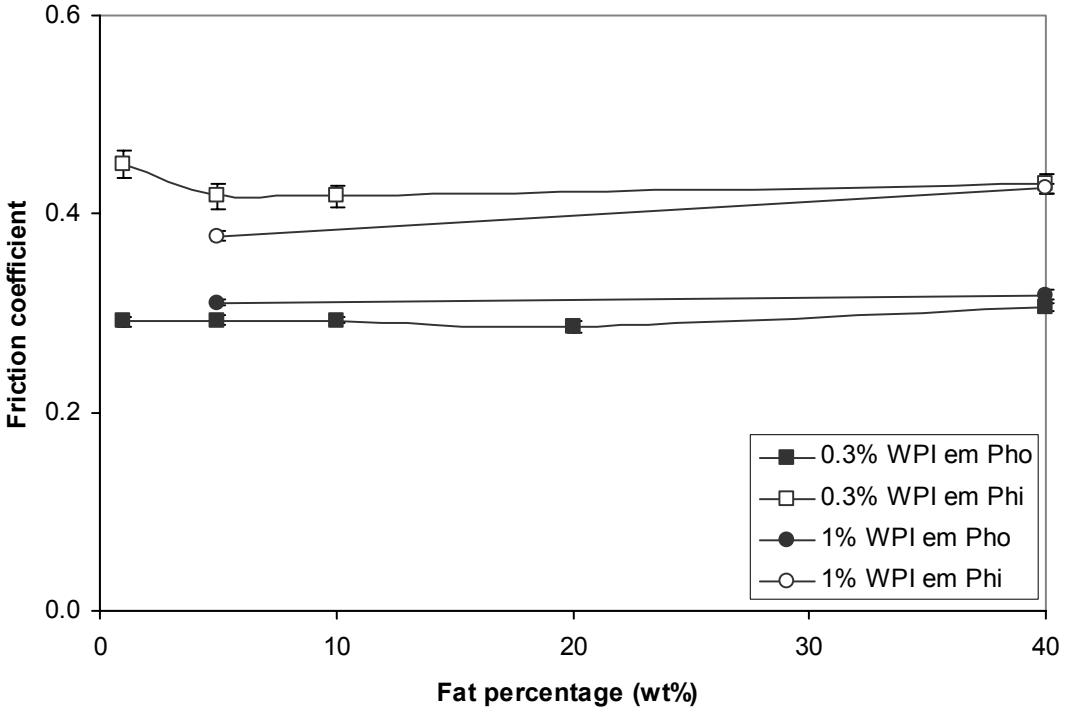

Figure 4.9: Friction coefficient as function of fat percentage of emulsions sheared at 0.08 m/s: 0.3 wt% WPI 40 wt% SF —■— and 1 wt% WPI 40 wt% SF—●— between hard hydrophobic PDMS (closed symbol) and hard hydrophilic PDMS (open symbol). Roughness is RH

Next, we investigate whether there is a lower limit to the amount of fat needed to form a lubricating layer, by decreasing the fat content to even lower values. The effect of surface hydrophobicity and emulsion stability was also studied. Figure 4.9 shows that even an emulsion with a fat percentage of 1 wt% lowers the friction just as efficiently as a 40 wt% emulsion, independently of surface hydrophobicity. Since shear-induced coalescence depends on the oil content, this observation gives further evidence that the reduction in friction is mainly due to oil deposition as a result of surface-induced coalescence. Also note that the least stable emulsion, 0.3% WPI, lubricates a hydrophobic surface better than the stable, 1% WPI, emulsion, again confirming earlier findings (Figure 4.6) and strongly suggesting the importance of surface-induced coalescence. For a hydrophilic surface the reverse is the case. The difference possibly lies both in the extent

of surface-induced coalescence, and in a difference in protein adsorption for the two surfaces, to which we will return shortly.

Viscosity of the oil

We consider the influence of the viscosity of the dispersed phase on the friction of food emulsions under mouth-like conditions. Even though the viscosities of the dispersed phases are highly different, the viscosities and droplet size distributions of the emulsions were nearly the same.

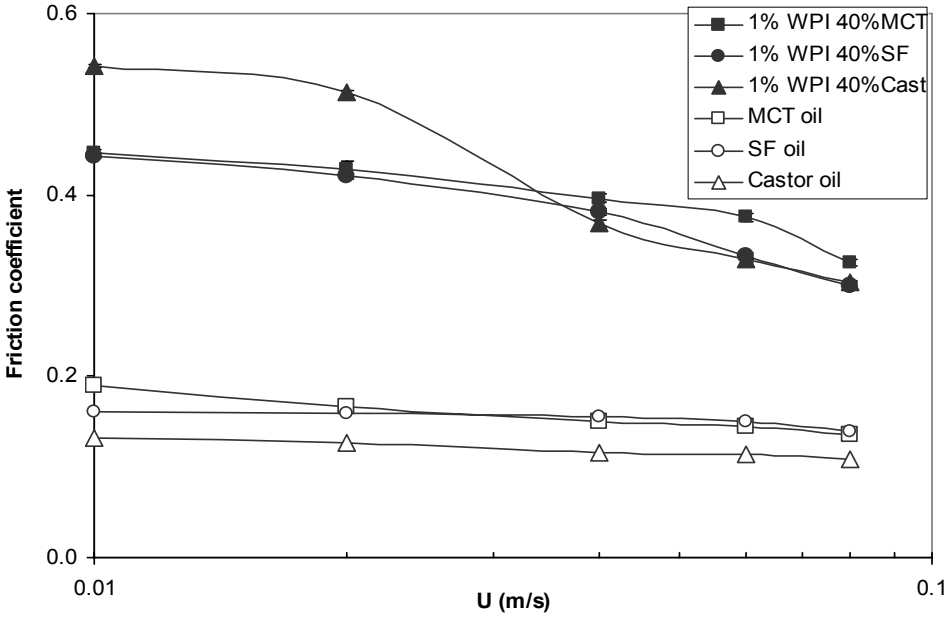

Figure 4.10: Friction coefficient as function of speed with 1 % WPI emulsified oils differing in viscosity (closed symbols) —■— MCT oil (low viscosity), —●— SF oil (medium viscosity), —▲— Castor oil (very high viscosity) and non-emulsified oils (open symbols), sheared between glass and hydrophobic hard PDMS (RL).

The results in Figure 4.10 show that in all cases, the friction for emulsified oil is much higher than for non-emulsified oil. The expected decrease of emulsion friction with oil viscosity is not observed. Instead, the emulsion containing the highly viscous Castor oil gives a higher friction in the low speed regime but shows a sharp decrease in friction in the high-speed regime, indicating mixed lubrication of the surfaces. On the other hand, judging from the shape of the partial Stribeck curve, the emulsions with the less viscous oils are more in the boundary regime throughout the whole speed range covered. These stable (1%WPI) emulsion droplets are not expected to spread on the surface and, also due

to their size, a difference in deformability (as a result of variation in oil viscosity) is not reflected in the friction data. In other words, viscosity of the dispersed phase hardly changes the character of emulsion lubrication for droplets around 1 μm.

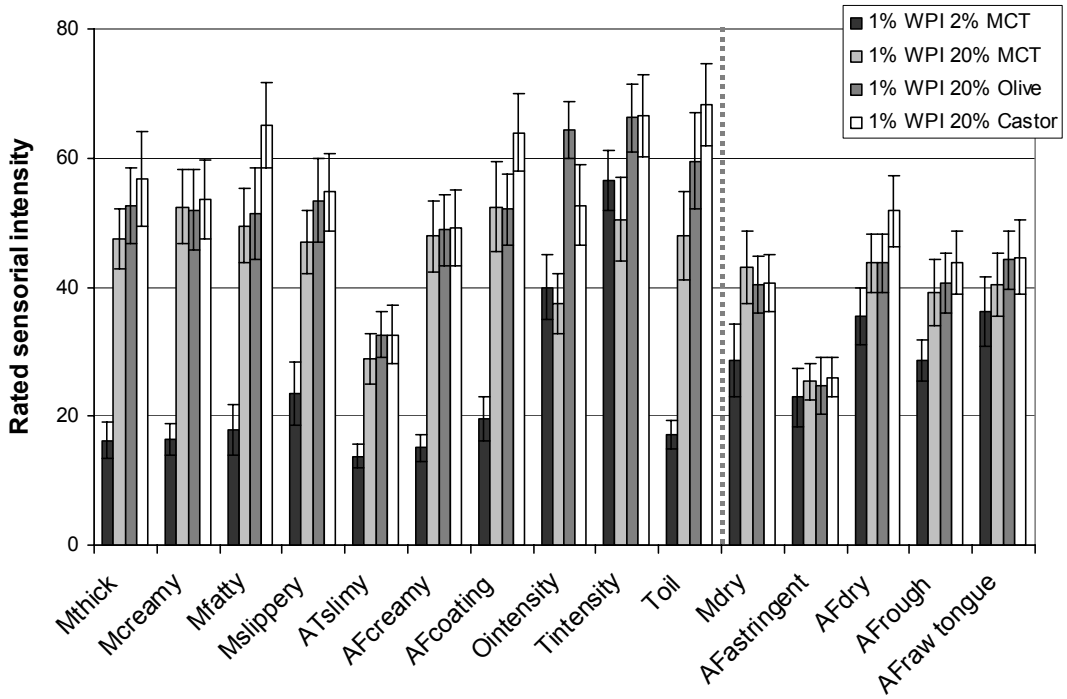

Figure 4.11: The rated sensory intensity at the different attributes on emulsions varying in dispersed phase from low viscosity oil MCT to high viscosity Castor oil. Error bar indicates standard error. Olive oil has a viscosity comparable to sunflower oil. The sensory attributes on the left are fat-related and on the right friction-related. M= mouth-feel, AF = after-feel, O= odour/aroma, AT= after-taste and T= taste attributes.

To see whether the difference in oil type does have a sensorial effect, the emulsions with a similar composition as in the friction experiments were sensorially evaluated using a trained Quantitative Descriptive Analyses (QDA) panel. Besides viscosity also the amount of oil was varied. Figure 4.11 shows that the high viscous Castor oil emulsion receives the highest scores on fat-related attributes such as thickness, fattiness, slipperiness, but not on creaminess. On the other hand, we do not find the inverse relation between perceived oral friction (AFrough, AFdry, AFraw tongue) and viscosity of the oil phase as we expected. In contrast, we find a positive relation between oral friction and viscosity. However, overall the sensory results did not show a significant difference in perception as function of viscosity of the dispersed phase, indicating that we can only deduce trends. This absence of a clear viscosity effect is in agreement with the

friction results (Figure 4.9). Presumably, the morphology of the tongue and the speed of entrainment between tongue and palate is such that also here no coalescence occurred with the stable emulsion, and thus no lowering of the friction was perceived. In other words, due to the stability of the emulsions and the undeformability of the droplets no effect of viscosity on oral perceived friction has been found. Illustrative for the finding that emulsion stability is essential for lowering the friction are the following results on a low fat emulsion (2% MCT). As expected, lowering of the fat percentage resulted in a dramatic decrease in perception of fat-related attributes. However, the low fat emulsion was also perceived as lowest in friction (Mdry, AFrough, AF raw tongue), which is in contrast with earlier findings (see Chapter 3) that the emulsion with the lowest friction is also perceived as most fat. Apparently, when emulsions are stable, a 2% MCT oil emulsion is not very different from a 20% MCT oil emulsion, in terms of perceived roughness, the 2% MCT oil even being perceived as lower in friction than the 20% MCT oil. In other words, the *stability* of an emulsion seems to determine the mouth-feel perception of fat. This indicates that an emulsion can be perceived as 'fatty' due to, most likely, aroma release but will only be perceived as 'creamy' if an oil layer is formed on the oral tissue, thereby lowering the perceived friction.

4.3.4 Salivary and bulk proteins

An important aspect that needs to be considered in understanding food emulsion lubrication is the influence on friction of an important component of these emulsions: proteins. Comparing emulsified oil with non-emulsified oil (Figures 4.4 and 4.10), or comparing protein solutions with emulsions and/or water (Figure 4.6) on hydrophobic PDMS we found already several indications that protein adhesion can strongly increase the friction. On the other hand, we also found examples in which protein adherence presumably facilitates emulsions lubrication, like in the case of lubrication of hydrophilic PDMS (Figure 4.9), a surface on which in absence of proteins oil droplets would not spread. Considering these previous indications and literature found on this subject (see § 4.1) we investigated the influence of protein content in the continuous phase (bulk proteins) on emulsion lubrication. We also consider the influence that salivary proteins can have on lubrication and on emulsion lubrication. The latter is of importance since

mouth surfaces are naturally covered with salivary proteins and we ultimately aim at understanding in-mouth lubrication.

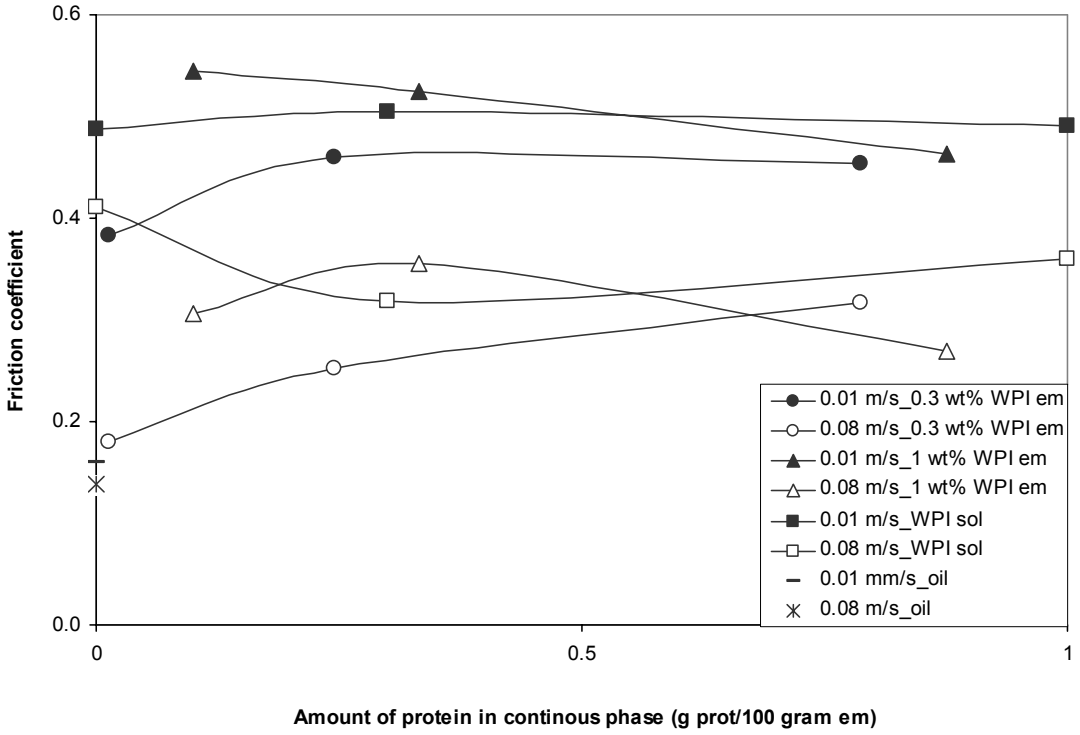

*Figure 4.12: Friction coefficient as function of protein concentration in the continuous phase of two emulsions: 0.3 wt% WPI 5 wt% SF —●— and 1 wt% WPI 5 wt% SF —▲— and concentration protein in WPI solutions —■—. Oil as comparison (- and *). Closed symbols speed of shearing 0.01 m/s, open 0.08 m/s; Sheared with hard hydrophobic PDMS-RL.*

In order to determine what the influence is of bulk proteins on lubrication by an unstable 0.3 wt% WPI stabilised emulsion and a stable 1 wt% WPI stabilised emulsion we measured friction coefficients as a function of estimated protein concentration in the continuous phase (assuming 2 mg/m^2 surface load [16]). First of all, Figure 4.12 shows that there is no linear relation between bulk protein concentration and measured friction over the protein concentration regime used. Secondly, Figure 4.12 reveals that the relation between friction coefficient and protein concentration is different for the different emulsions (stable vs. unstable). This implies that unstable emulsions lubricate the surface better than the stable emulsion, independent of the bulk protein concentration. Furthermore, Figure 4.12 shows that protein solutions always give rise to a higher friction force than the 0.3% WPI stabilised emulsion indicating that the presence of fat or particles does have an effect on the friction. In short, the difference in measured friction between

shearing a 0.3 wt% stabilised emulsion and a 1 wt% stabilised emulsion is the result of the combined effects of protein adherence and surface-induced coalescence, in which oil spreading due to surface-induced coalescence is the main effect.

In view of the in-mouth conditions relevant for the sensory perception of emulsions, we also investigated the influence of salivary proteins. The tongue is a hydrophobic surface and since saliva is the natural lubricant covering the tongue, saliva is expected to lubricate the hydrophobic PDMS surface quite well. Surprisingly, the results in Figure 4.13 show that saliva gives a much higher friction than both water and emulsion. This is remarkable since saliva largely consists of water, and furthermore, a low friction is expected with the main biological lubricant of the mouth, saliva, present in the tribological contact. Apparently, saliva components, such as the mucin glycoproteins, behave similar as bulk proteins (Figure 4.12) regarding adherence and increasing the friction. When the glass and PDMS surfaces were coated with saliva and the emulsion was sheared between the coated surfaces the friction is increased in comparison to the emulsion alone, and slightly lowered in comparison to saliva alone. In summary, adherence of proteins from either the emulsion, saliva or both largely increase the friction.

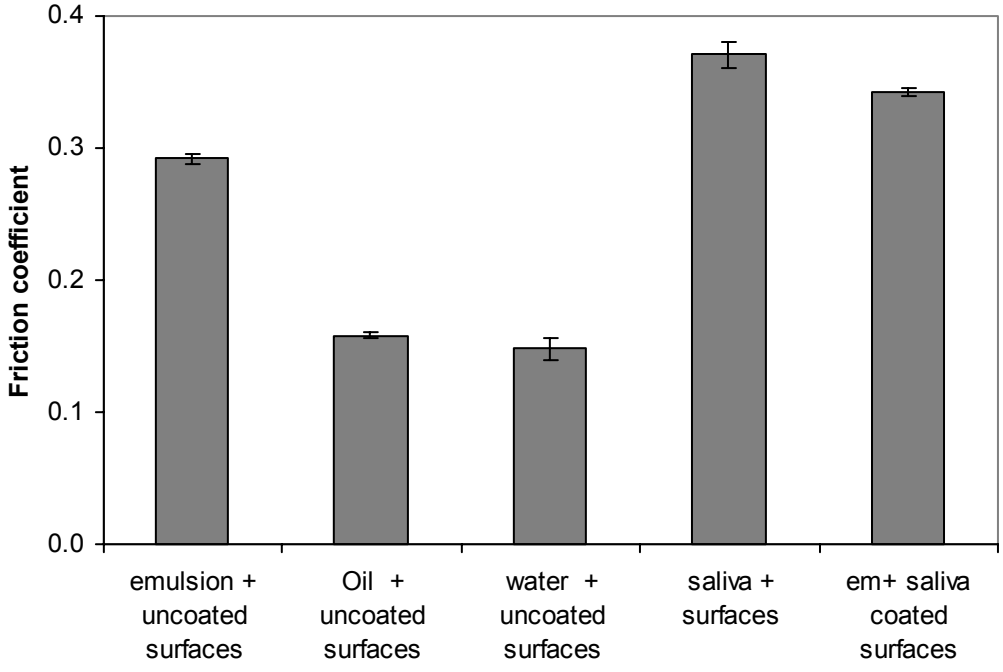

Figure 4.13: Friction coefficient measured at 0.08 m/s between glass and either uncoated or saliva-coated hydrophobic hard PDMS (RH) with emulsion (0.3 wt% WPI 10 wt% SF) as lubricant compared with SF oil, water, and saliva as lubricants.

4.4 Discussion

With the ultimate aim of understanding fat perception, in this work we have studied lubrication by protein-stabilised emulsions under in-mouth conditions. To our knowledge, no study has been performed so far that addresses the regime of oral lubrication by food emulsions. Malone and co-workers [4] did find correlations between sensory perceived slipperiness and friction results in the mixed regime using biopolymer solutions as lubricant. However, different from Malone's, our set-up makes a parallel sliding movement instead of circular, we use spherical rough surface on a flat smooth surface instead of very smooth ball (which rolls instead of slides) on a smooth surface and our applied load is lower. Moreover, we use protein-stabilised unthickened food emulsions as lubricant instead of surfactant stabilized thickened emulsions. Identifying the regime of lubrication is the essential first step in understanding oral lubrication. We argue that in-mouth lubrication of food emulsions is in the boundary/mixed regime, implying that viscosity of the non-thickened emulsion is often not the most important characteristic, but rather the ability of the emulsion to interact with the surface. We clearly show that under the mouth-like conditions applied in our tribometer (OTC) modified PDMS is not hydrodynamic, but boundary and/or mixed lubricated using the separate Newtonian liquids oil and water as well as emulsions (Figure 4.2 and 4.4) as lubricants. Note that due to the limited speed range the OTC covers, we can not distinguish whether altering the surface and/or lubricant parameters result in a shift within the Stribeck curve or in a shift of the Stribeck curve.

While there is essentially no previous literature on how food emulsions lubricate the mouth, there are some relations with findings on hydrodynamic emulsion lubrication in the context of industrial processing of e.g. metal. In these applications, boundary/mixed lubrication is usually avoided. On the other hand, the boundary/mixed regimes are often crucial in the field of bio-lubrication, e.g. in joint and prosthetic materials lubrication [17-22]. Below, we discuss some of the relevant papers from the areas of hydrodynamic emulsion lubrication and bio-lubrication, and relate them to the work presented here.

One of the questions we have tried to answer is how emulsions lubricate surfaces. In industrial processing of metal, o/w emulsions are widely used as lubricant in the

hydrodynamic regime between hard contacts. However, the mechanism of lubrication by o/w emulsions is still not yet fully understood. As a consequence, the design of lubricious emulsions is still quite empirical [6]. In their study focussed at understanding emulsion lubrication, Cambiella and co workers [6] found that interaction between the surfaces and the oil droplets determines the mechanism of lubrication and that this interaction is primarily controlled by the emulsifier concentration, and thus also by the emulsion stability. Consistent with this, Dubey et al [7] reported an inverse relation between emulsion stability and lubrication in their experiments on cold rolling of steel. As described in Chapter 3, we also found that emulsions, which are more sensitive towards coalescence are better lubricants. Indeed, the present work confirms the importance of emulsion stability for lubrication (Figure 4.6). We also found more indications that surface-induced coalescence is an important mechanism in lowering the friction (as we argued before in Chapter 3). In other words, friction is determined by oil covering the contact points and/or oil film formation due to oil release from the emulsion.

An important finding is that the friction for unstable emulsions is always well above the friction of non-emulsified oil. This points to additional sources of friction, most likely, layers of proteins in the lubrication contacts. This is an issue that also arises in the field of bio-lubrication, where it has been shown that proteins adhere to surfaces and influence friction in the boundary and mixed regime by changing adhesion between the surfaces. Sibarani and co-workers found that proteins adhere more at hydrophobic surfaces (such as PDMS) than at less hydrophobic surfaces (such as Polyvinlychloride, PVC) [23]. In contrast to this, Heuberger et al [17] and Widmer et al [22] reported reduced protein adherence at hydrophobic surfaces in comparison to hydrophilic surfaces. Furthermore, they report that adherence of proteins, in general, increases boundary friction and the largest increase in friction is found at hydrophobic surfaces. Karuppiah [19] et al also found an increase in boundary friction as function of protein concentration. However, they report, using the same surface as Widmer et al [22], a higher friction on hydrophilic UHMWPE (ultra-high molecular weight polyethylene used in total joint replacements) surfaces instead of on hydrophobic UHMWPE. Our friction experiments confirm the observed higher friction as function of protein concentration (Figure 4.12) on hydrophobic PDMS. In addition, Malone and co-workers found an increase in sensory

perceived oral friction (astringency) as function of adhered amount of heat-treated milk on a mucin-coated surface, which implies that adherence of protein can have a sensory effect.

However, in-mouth lubrication by food emulsions is certainly not determined by protein-induced friction alone: we have clearly shown that the presence of oil lowers the friction. But how much oil is needed for this effect? The amount of oil necessary to lower the friction has been addressed by De Hoog et al [24]. They found that there was no effect of oil content in the regime 10-40 wt% oil. Also Malone and co-workers [4] varied the fat content of their surfactant-stabilised emulsions. They did find an effect of fat in the mixed regime between 1-15 wt% and so did de Wijk and Prinz [5] with their custards varying in fat content. However, Malone and Prinz used smooth rubber surfaces and thickened emulsions whereas De Hoog used unthickened emulsions and rough oral surfaces. In the present work, we lowered the oil content further to 1 wt% and found that for both stable and unstable emulsion there is still no effect of oil content, which is for unthickened protein-stabilised emulsions, in agreement with De Hoog [24]. In other words, only a very small amount of oil is sufficient for lowering the friction. Given the fact that there is an inverse relation between fat perception and friction [1-5], these findings contradict the sensory findings that fat content very clearly has an effect on fat perception [4; 25-27] and therefore on friction.

Deformability of emulsion droplets is also expected to influence emulsion lubrication. Vicente and co-workers suggested in their work that at a high viscosity ratio between dispersed and continuous phase the droplets become undeformable and are forced into the contact zone at high speeds, resulting in coalescence of the droplets [9]. Note that their emulsion droplets are not stabilised by either surfactant or protein and are larger, and therefore more deformable than the droplets used here. Considering the work described in Chapter 3 on the effect of coalescence on perception, this would indicate that high viscosity dispersed phases (viscous oil) lower the perceived oral friction and thus increase the perception of fat related attributes such as creaminess. Sensorial analyses indicate that the small undeformable droplets have hardly any influence on perception and the perceived friction is increased instead of decreased. There are some indications that there is a relation with viscosity of the dispersed phase and perception of fat-related attributes such as fatty, thick, but not with creamy. Most likely the emulsions used were

not sensitive towards coalescence, and therefore the effect of viscosity of the dispersed phase was minor.

Next, consider the wider implications of our work for understanding sensory fat perception. In the mouth, emulsions come into contact with a rough, hydrophobic and highly deformable surface, but also with saliva. Our results show that just like for emulsion lubrication, the presence of salivary proteins enhances the friction due to adherence of proteins to the surface. Interaction of saliva with an emulsion slightly lowers the friction, but still the friction is higher than with emulsion alone. In a different set-up Ranc [28] and co-workers coated a pig's tongue with saliva, dried the tongue and film and measured under these dry conditions the friction using a steel ball. In comparison to an uncoated dry tongue, the coated tongue gave rise to a lower friction. The lower friction in that case could be due to the presence of a sacrificial boundary layer [29], whereas in our case saliva in its hydrated form can behave like a gel allowing boundary sliding between two interfacial salivary films similar to what is proposed by Hsu & Gates [29].

Is influencing friction the only role saliva plays in in-mouth lubrication? Saliva consists mainly of glycoprotein, mucins, which are highly surface-active and can form stable emulsions in the presence of oil [30]. Experiments conducted with non-emulsified fat in the mouth show an extensively increased saliva production upon consumption (data not shown) in comparison to emulsified fat. After spitting out the non-emulsified oil, a stable emulsion is formed. This indicates that by producing saliva and thus exposing the free oil to the surface-active glyco-proteins the hydrophobic tongue surface is cleaned very efficiently by creating an emulsion. The cleaning of the surface by saliva would also explain why high-weight fractions of oil do have an effect on in-vivo sensory perception but not on in-vitro friction experiments. High amounts of fat form a reservoir of lubricant counteracting the saliva cleaning-activity and in that way they prolong the 'fat' sensation.

Based on the present work, we can say that the mouth is boundary/mixed lubricated and thus that surface characteristics and interaction of the lubricant with the surface play an important role. Emulsions, which are sensitive towards surface-induced coalescence, containing a minimum amount of free protein, are most efficient in reducing in-mouth friction (boundary/mixed) and thus in enhancing fat perception. There is no effect of emulsion fat content (in the range 1 wt% - 40 wt%) on friction. On the other

hand, high amounts of oil do allow for the prolonged constant formation of such thin lubricating layers, and this may explain why the amount of fat is nevertheless important in fat perception.

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5

Fat retention at the tongue and the role of saliva: adhesion and spreading of stable versus unstable emulsions

Abstract

Fat perception of food emulsions has been found to relate to in-mouth friction. Previously, we have shown that friction under mouth-like conditions strongly depends on the sensitivity of protein-stabilized emulsion droplets to coalescence. Here, we investigated whether this also implies that oral fat retention depends in a similar manner on the stability of the emulsion droplets against coalescence. We investigate the separate contributions of droplet adhesion and droplet spreading to fat retention at the tongue, as well as the role of saliva.

We performed *ex vivo* (Confocal Raman Spectroscopy; Confocal Scanning Laser Microscopy) experiments using pig's tongue surfaces in combination with human *in vivo* experiments. These revealed that unstable (protein-poor) emulsions are retained more at the tongue than stable (protein-rich) emulsions. Furthermore, the layer formed by adhering protein-poor droplets is more stable against rinsing. Saliva is found to be very efficient in removing fat and emulsion droplets from the oral surface but its role in fat retention need further research. We relate our results to the colloidal forces governing droplet adhesion and spreading.

D.M Dresselhuis, M.A. Cohen Stuart, G.A. van Aken, R.G. Schipper, E.H.A. de Hoog

5.1 Introduction

In connection to increasing awareness concerning obesity, there is great public and scientific interest in designing food emulsions that contain less fat, but nevertheless give a full-fat sensation. Several authors suggested that, besides aroma release and bulk viscosity, the ability of the emulsion to act as an in-mouth lubricant is important in fat perception [1-5]. In Chapter 3 we confirmed this relation between sensory fat perception and perceived friction. Our recent work also highlights that emulsion stability is important in determining friction. We demonstrated that emulsions more sensitive towards coalescence were not only more efficient in lowering oral perceived friction, but also in reducing the measured friction using both pig's tongue and a modified PDMS as a model human tongue surface (Chapter 3 & 4). An increased sensitivity towards coalescence was, amongst others, accomplished by reducing the interfacial protein concentration of the emulsions. Moreover, we showed that under mouth-like conditions friction is, to a large extent, governed by the interaction of the emulsion components with the surfaces (Chapter 4).

Both for the model tongue surfaces as well as for the actual human tongue it is unclear whether adhesion of emulsion droplets alone already lowers the friction or that subsequent spreading of emulsion droplets on the surface is necessary. For the PDMS model surface we have found that the friction is increased when saliva is added to the protein-stabilised emulsions lubricating the surface. This raises the question what the influence of saliva is on adhesion and spreading of emulsion droplets on the tongue surface and thus on fat retention.

Besides saliva, oral surface characteristics can also influence fat retention. In the mouth, emulsion droplets come into contact with a highly heterogeneous tongue surface covered with different types of relatively large papillae (≈300 μm). Among the papillae, the filiform papillae have keratinised tips giving the tongue its roughness (see Chapter 2). The tongue is intrinsically hydrophobic, but hydrophilic when covered with saliva (see Chapter 2). Food emulsion droplets are often on average two orders of magnitude smaller in size than the papillae.

Ultimately, we would like to be able to explain adhesion and spreading of protein-

stabilised emulsion droplets on the highly complex surface of the tongue in terms of colloidal forces. Besides the complexity of the tongue surface, many other factors make accurate predictions of droplet adhesion and spreading in terms of colloidal forces difficult. For example the nature of the adsorbed layer (thickness, surface charge, surface hydrophobicity) depends in a complicated way on protein concentration and other conditions (e.g. pH, salt, temperature) during emulsification. Another complicating factor is the presence of free proteins and surface active mucins [6] and other saliva components in emulsions in the mouth. These are expected to both be capable of affecting droplet adhesion through adhesion to the tongue and interaction with the emulsion droplet surfaces.

Adsorption of saliva components and bulk proteins from the emulsions on the tongue is indeed to be expected: the tongue is highly hydrophobic and both free protein and saliva components are known to adhere to hydrophobic surfaces [7-9]. Saliva is a complex biological fluid, which consists of water, electrolytes, small organic compounds and numerous proteins of which the mucins (glycoproteins with sialic acid side chains) are most predominantly present.

Interaction of saliva components with emulsion droplets was considered by Silletti *et al*. [10]. These authors performed *ex vivo* experiments, which revealed that saliva at pH 7 can interact with emulsion droplets via electrostatic interaction or depletion, depending on the charge on the emulsion droplets. Shi *et al*. [6] showed that mucins can be very effective in stabilising o/w emulsions, and in connection to that we argued previously that saliva is involved in removal of fat from the tongue (Chapter 4).

In the present work, we want to connect the conclusions from our previous work on lubrication of model surfaces (pig's tongue and modified PDMS) by protein-stabilized emulsions, to fat retention on the tongue. In particular, we want (based on earlier work) to verify whether emulsions, which are more sensitive to coalescence, indeed are retained in greater amounts and more strongly on the tongue. Furthermore, we address the separate roles of droplet adhesion and droplets spreading in oral fat retention, and the role of saliva in modifying these processes. Combining *in vivo* and *ex vivo* experiments and using different techniques allows us to independently study various aspects of fat retention on the tongue. Finally, we also discuss to what extent our results can be explained in terms of

the colloidal forces that govern droplet adhesion and spreading on the surface of the tongue.

5.2 Material and methods

5.2.1 Emulsion preparation and characterisation

O/W emulsions containing 40 wt% oil were prepared according to the procedure described in Chapter 3, but in this case no vanilla, sucrose or salt were added. The emulsions were stabilised by either 1 wt% or 0.3 wt% Whey Protein Isolate (WPI; BiPro, Davisco, USA). Sunflower oil (SF; fully winterized, η= 60mPa.s, kindly provided by Cargill Amsterdam, The Netherlands) was used as the oil phase. The emulsions were diluted with the continuous phase to obtain a 10 wt% SF oil emulsion (or a 7 wt% SF oil emulsion in case of the *in vivo* experiments). The 1 wt% WPI stabilised emulsions were previously shown to be stable against coalescence in the mouth, whereas the 0.3 wt% WPI stabilised emulsions were shown to be unstable under the same conditions (see Chapter 3).

Table 5.1: Average droplet size and viscosity (at $51s^{-1}$) of the emulsions

	Average droplet size		Viscosity
	D[3.2] μm	D[4.3] μm	$51s^{-1}$ mPa.s
1 wt% WPI 7 wt% SF	0.92	1.46	1.6
1 wt% WPI 7 wt% SF **L**	94.24	108.60	2.9
0.3 wt% WPI 7 wt% SF	1.15	1.83	1.4
1 wt% WPI 40 wt% SF	0.93	1.42	6.4
0.3 wt% WPI 40 wt% SF	1.10	1.73	6.0

The average particle size was determined by light scattering (Mastersizer 2000, Malvern, Worchestershire, UK) (see Table 1). Note that the 7 wt% oil and 10 wt% oil emulsions had similar average particle sizes. The rheology of the emulsions was analysed in duplicate at 20°C using a Physica MCR 300 Rheometer (Anton Paar, Graz, Austria) with a cone-plate geometry with a diameter of 75 mm, a 1º angle and a gap of 50 μm (see Table 1). No clusters or aggregates were observed with light microscopy. In addition a 1 wt% emulsion (7 wt% SF) with large droplets (L) instead of small droplets was obtained by mixing SF oil and a protein solution with an ultraturrax (T25 basic, IKA, Staufen, Germany).

5.2.2 *In vivo* fat retention

Five healthy subjects processed non-emulsified sunflower oil as well as emulsions (listed in Table 1) using a standardised procedure. No creaming of emulsion droplets was observed during the time of the experiments. The procedure was as follows: the subjects had not eaten and drank for at least 2 hours before the experiment. Before starting the experiment the subjects rinsed their mouth twice for 15 s with 10 ml of tap water to clean their mouth. The subjects were unaware of the type of emulsions served to them. They processed 10 ml emulsion for 15 s moving the tongue against the palate with a frequency of 1 Hz and spitting it out in a clean cup. Swallowing was not allowed during the whole procedure. Immediately after, the subjects rinsed their mouth with water for 15s using the same procedure as for the emulsion. This rinsing step was repeated once more after which the subjects had 5 minutes of rest in which they were allowed to swallow and speak. This rest period was enough to completely remove all fat from the tongue before the next experiment. The cups containing 10 ml of emulsions and 10 ml of rinse (tap) water, as well as the cups the subjects spat in, were carefully weighed before filling them and after emptying them to calculate exactly the amount the subjects processed and subsequently spat out. The rinse water and the unprocessed and processed emulsions were analysed with a gravimetric method (Röse Gottlieb, IDF 16C:1987) by a certified lab (COKZ, Leusden, the Netherlands) within a day to quantify the amount of fat.

In order to follow the fat retention in time, a slightly different method was applied in which the rinse step after processing the emulsion was delayed for 1, 2, 5 or 20 min after the emulsion was ingested. During this delay swallowing was allowed, but speaking was not. This experiment was performed by 2 of the 5 subjects. We assumed that after ingestion and spitting out the *same* emulsions (so for every time point) the adherence of the droplets to the surface is equal. This means that the decrease in amount of fat in the mouth is due to swallowing of saliva containing fat residues.

5.2.3 Saliva and tongue preparation/characterisation

Tongue samples of freshly slaughtered 4 months old pig's were prepared according to the method described in Chapter 2 and stored at -80°C. Immuno-histochemical techniques, as described by Schipper *et al.*[11], were used to verify whether there were

still saliva remnants on the tongues of the pig's after this preparation. In short, we used antibody staining for one of the mucins present in saliva, MUC4 [12]. Tissue samples were taken at the back and the middle part of the tongue and fixated for two hours using the Carnoy tissue fixative (6:3:1 mixture of absolute ethanol, chloroform and acetic acid). This fixative is regularly used in mucosa characterisation (e.g. [13; 14]). To evaluate the effect of rinsing and freezing, the samples were: 1) not rinsed and directly fixated in Carnoy 2) first rinsed with hand warm water, then fixated in Carnoy 3) rinsed, snap-frozen in N_2, stored at minus 80°C for less than 3 weeks, defrosted (following the method described in Chapter 2) and then fixated in Carnoy. Next, the tongues were sliced longitudinally at regular intervals of 5 mm width, cleared in xylene, and embedded in paraffin. Serial paraffin sections of 4 μm thickness were prepared and incubated for 90 minutes with the MUC 4 antibody (Zymed Labs , San Francisco, CA) diluted in 1% PBS-1% BSA. Secondary antibody-HRP (Dako Envision System) was incubated for 30 minutes, and samples were developed using diaminobenzidine and then counterstained with hematoxilin-eosin. The tissue staining was evaluated using a light microscope.In all our experiments we used pooled unstimulated saliva of 30 healthy persons, which was collected following the procedure of Silletti *et al*.[10]. From the pig's tongue, the middle part was used, which is the part involved in swallowing.

5.2.4 Raman spectroscopy

A Confocal Raman Spectroscope (Model 3510 Skin Composition Analyzer, River Diagnostics, Rotterdam, The Netherlands) specially adapted for following moisture uptake from skin creams in skin, was used to analyse the presence of fat at pig's tongue. Here, 20 μl of emulsion, sunflower oil, protein solution or saliva was applied to prepared tongue samples taken at the middle part of the pig's tongue, making sure that all the space between the papillae was filled with emulsion. Two minutes after the emulsion was applied to the tongue it was rinsed during 10s with a non-pulsating flow at two different flow rates (9.5ml/min vs. 76 ml/min). The reduction in amount of retained fat was measured by determining the ratio between retained fat (intensity of Raman shift at wave number 1658 cm^{-1}) at the papillae surface before and after rinsing. Note that a 'clean' tongue did show a Raman shift at 1652 cm^{-1}, but not at 1658 cm^{-1}. The confocal unit in

combination with video analysing technique allowed positioning of the laser on the papillae surface and thus enabling measuring fat present at the surface. Several spots at the papillae surface are monitored and the intensities are averaged. The effect of saliva on fat retention was determined by incubating the pig's tongue for 2 minutes with 20 µl of unstimulated human saliva and subsequently removing the excess of saliva and applying 20 µl of emulsion. Evaporation of saliva was avoided. Two different emulsions were used, a 1 wt % WPI 40 wt% SF (stable) and a 0.3 wt % WPI 40 wt% SF (unstable) emulsion.

5.2.5 Confocal scanning laser microscopy

Adhesion of intact emulsion droplets to pig's tongue was determined by applying emulsions differing in stability (1 wt% WPI vs. 0.3 wt% WPI) and stained with both the fat soluble fluorescent dye Nile red (Invitrogen, the Netherlands) as well as the dye with affinity for protein, FITC (Invitrogen, the Netherlands), to a pig's tongue. Nile red was added to the oil phase before emulsification. The same procedure as described for Raman spectroscopy (see §5.2.4) concerning rinsing and applying saliva was used. The presence of emulsion droplets was monitored using 2 laser lines of a Confocal Scanning Laser Microscope (Leica Microsystems GmbH, Heidelberg, Germany). Nile red and FITC were excited with the 568 nm and with the 488 nm laser line, respectively. The samples were placed upside down and the images were taken, with an inverted CSLM, close to the glass plate, at maximum intensity. For every experiment a fresh piece of tongue sample was taken. Drying of the tissue during the experiment was minimized by creating a water saturated environment and by optimising the scan speed. Using a standardised image analysis protocol (ImageJ, Wayne Rasband, USA) the amount of red and thus amount of fat was compared before and after rinsing.

5.3 Results

First we present the results on *in vivo* fat retention and its dependence on the emulsion properties stability against coalescence, fat percentage and droplet size. Next, we evaluate the resistance of retained fat against rinsing and the natural action of saliva. Finally, we present our results on *ex vivo* fat retention, focussing also on the role saliva plays in droplet adhesion and droplet spreading on the tongue surface.

5.3.1 *In vivo* fat retention

Emulsions that are more sensitive towards coalescence are expected to spread more on the tongue surface than more stable emulsions. Indeed, Figure 5.1 shows that after oral processing of a less stable emulsion (0.3% WPI) more fat remains on the tongue surfaces than with the more stable emulsion (1% WPI). Concerning the stability of the layer formed on the tongue, we compared the amount fat remaining after spitting and after one time rinsing. Oral processing of the unstable (0.3% WPI) emulsion not only results in more fat on the tongue but the fat is also relatively harder to remove.

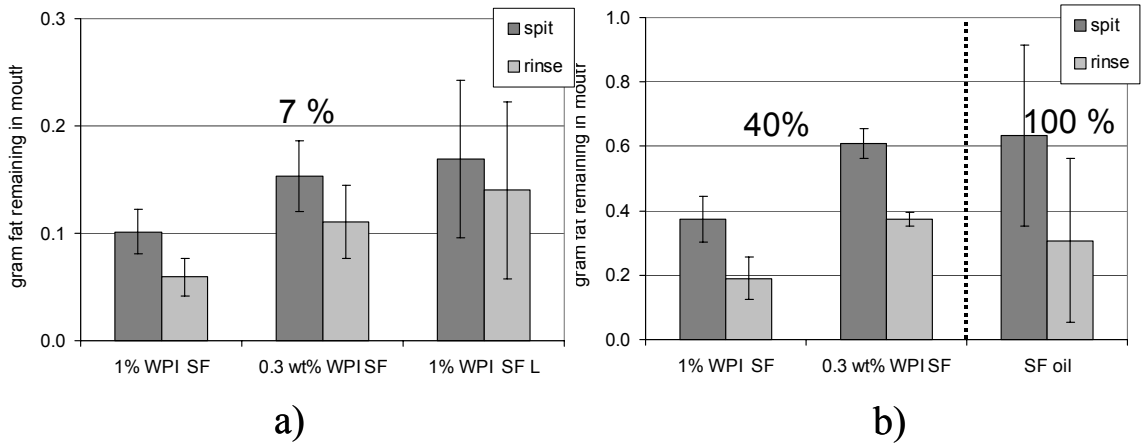

Figure 5.1: Average amount of fat remaining in mouth after oral processing emulsions, which vary in sensitivity towards coalescence, by 5 different subjects. The emulsions varied in fat content: a) 7 wt% b) 40 wt% fat and 100 wt% sunflower oil. D[3.2] around 1 mm, emulsion indicated with L has a D[3.2] ~ 94 mm

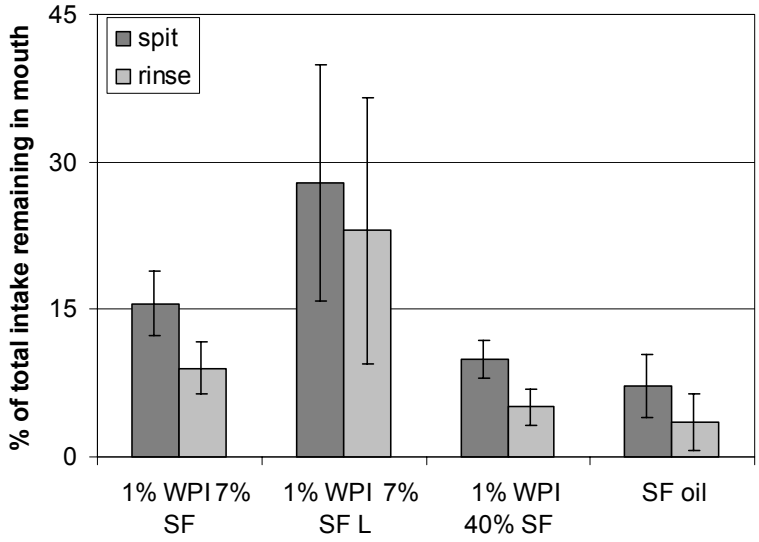

Figure 5.2: Average relative amount of fat remaining in mouth after oral processing emulsions by 5 different subjects. D[3.2] around 1 μm, emulsion indicated with L has a D[3.2] ~94 μm

Furthermore, Figure 5.1 shows that increasing the droplet size results in more fat retention on the tongue. This may be because droplet stability decreases with increasing droplet size. Increasing the fat content of an emulsion results in higher absolute amounts of fat remaining on the tongue, but the relative fat retention (grams retained fat per grams of emulsified oil intake) decreased.

This is further illustrated in Figure 5.2. The *absolut*e amount of fat remaining in the mouth *increases* from left to right, from 1% WPI 7% SF until 100% SF. However, apart from the large droplets, the *relative* amount of retained fat *decreases* from left to right. Note that of around 8.7 grams of pure sunflower oil only 0.6 gram remains in the mouth after expectoration. Therefore, a relatively large amount of fat is expectorated when processing 100% oil in comparison to e.g. an emulsion containing 7% oil.

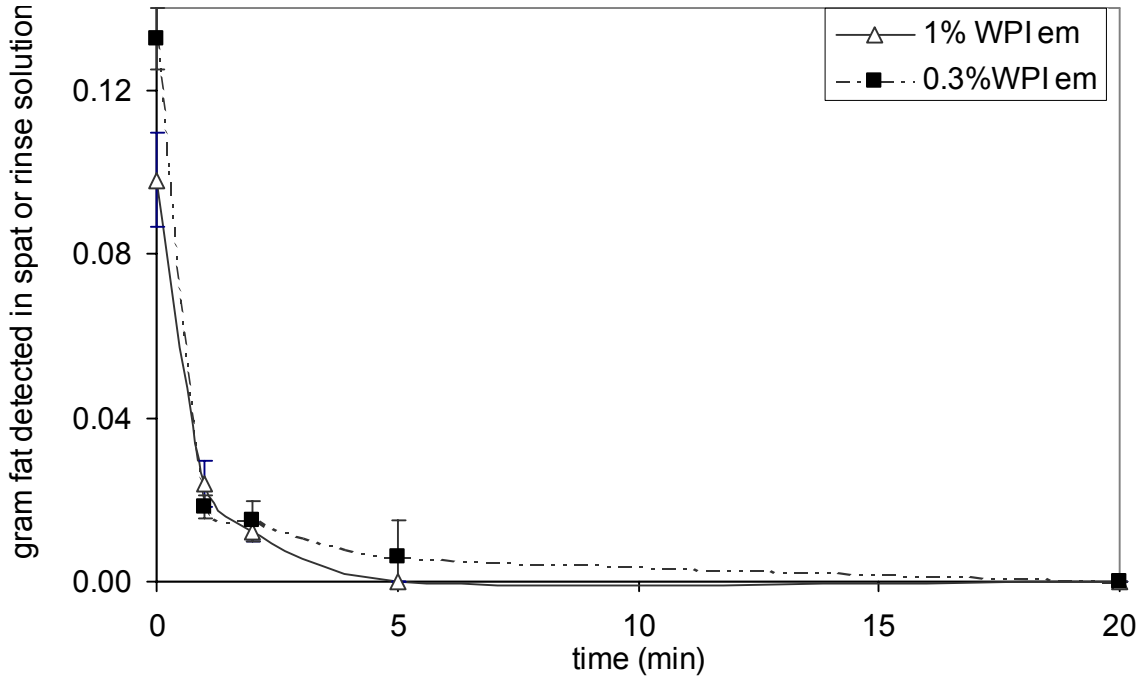

Figure 5.3: Natural decrease of amount of fat on oral surface in time by saliva after consuming a stable 1 wt% WPI 7 wt% SF and an unstable 0.3 wt% WPI 7 wt% SF emulsion.

Figure 5.1 showed that rinsing with water removed part of the amount of fat, but how effective is saliva in removing fat? Figure 5.3 shows that upon normal movement of the tongue, when swallowing is allowed, within several minutes the retained fat in the mouth, which can be rinsed off is drastically reduced. This could be due to saliva quickly washing away fat from the surface, which is then swallowed. In summary, emulsions that

are more sensitive towards coalescence give rise to a higher retention of fat at the tongue surface and this fat is harder to remove by rinsing with water (as compared to emulsions that are less sensitive to coalescence). While increasing the fat content of emulsions increases absolute fat retention, it decreases the relative amount of fat retained at the tongue.

5.3.2 The influence of saliva on fat retention

Our results in Figure 5.3 suggest that saliva is very effective in removing fat from the surface of the tongue. But what is the effect of saliva on fat retention and more specifically on adhesion (and spreading) of emulsion droplets on the tongue surface? In order to address this question, we used techniques that can detect fat on the molecular level (Confocal Raman Spectroscopy) and on emulsion droplet level (Confocal Scanning Laser Microscopy, CSLM).

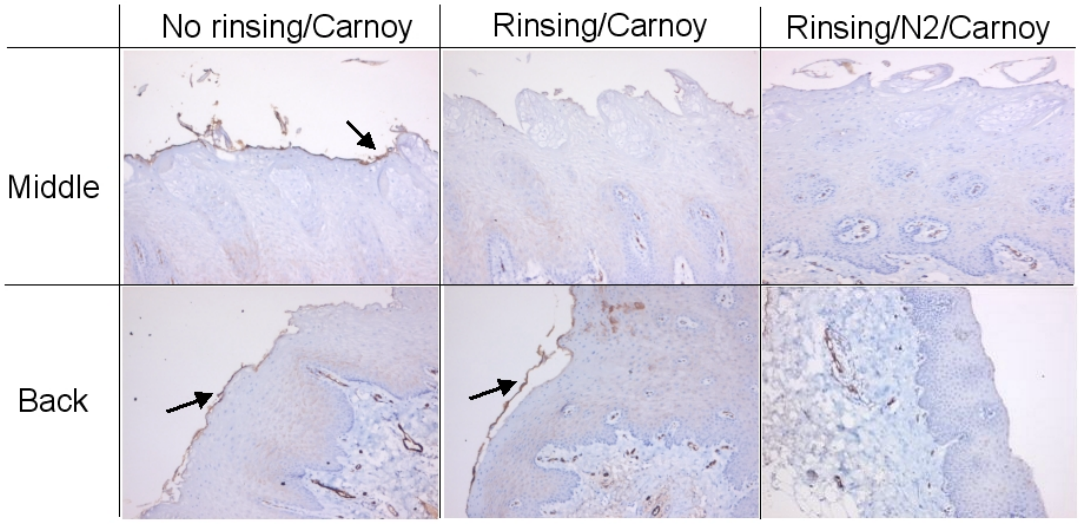

Figure 5.4: MUC4 antibody staining of samples taken at the back and the middle of the tongue prepared by 1) not rinsing the tissue, fixating in Carnoy, 2) Rinsing, fixating in Carnoy 3) Rinsing, snap-freezing in N_2, storing at -80℃, defrosting and fixating in Carnoy. The arrows indicate the presence of MUC4 on the surface (dark brown stain)

Saliva at the pig's tongue surface

The pig's tongue used in our experiments is rinsed with hand warm water before preparation and preservation as described in Chapter 2. In order to be able to study the effect of saliva separately from the interaction with saliva-free tongue surface, we verified whether there was still saliva, containing mucins, present after this rinsing step. This was

done using immunohistochemical techniques. Immunohistochemical staining with MUC4 antibody (dark brown colour; Figure 5.4) shows that initially mucins are present at the middle and at the back of the tongue but that rinsing removes them from the tongue surface (mainly from the middle part). This shows that MUC4 can be removed from the oral surface fairly easily by rinsing. MUC4 is also completely removed from the tongue by freezing and defrosting the tissue. MUC5b and MUC7 which are also present on the pig's tongue surface (Schipper *et al*. [11]) could not be detected at all after the rinsing and freezing steps (data not shown). In summary, the pig's tongue samples as used in our experiments are not covered with mucins. This allows us to study the difference between fat retention on human saliva covered oral surfaces as opposed to non covered oral surfaces, without interference of pig saliva mucin remnants.

Molecular fat detection

Figure 5.5 shows the Raman shift caused by light interacting with the different molecules present in the different solutions between and on the papillae. The C=C stretch is characteristic for the presence of fatty acids (wave number 1658 cm^{-1}).

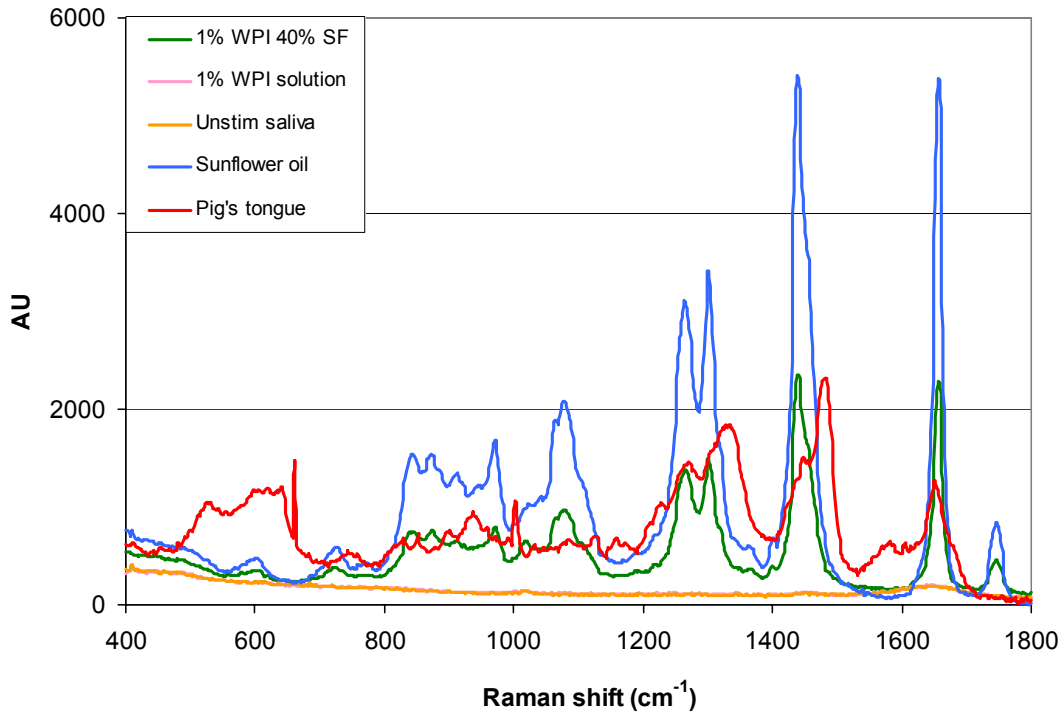

Figure 5.5: Raman shift spectra comparing 1% WPI 40% SF emulsion with 1% WPI protein solution, unstimulated saliva, sunflower oil and pig's tongue at the C=C stretch (1658 cm^{-1}). The spectra of unstimulated saliva and 1 wt% WPI solution superimpose.

Pig's tongue does show a peak at 1652 cm^{-1} but not at 1658 cm^{-1}, indicating that no fatty acids are naturally present on a clean pig's tongue. Unstimulated saliva and the protein solution also do not have a C=C stretch signal and because of the low concentrations of protein both the spectra superimpose. Hence we can use confocal Raman Spectroscopy to monitor specific fat retention at the tongue surface.

Figure 5.6 shows that rinsing the oral surface with water reduces the amount of fat on the tongue. In the case saliva is present, more fat is detected on the tongue after rinsing than when no saliva is present. However, this difference disappears when comparing fat residues (in presence and absence of saliva) after rinsing at a high flow. Apparently, emulsion droplets do interact with saliva, initially favouring fat retention. However, as is shown in Figure 5.4, the interaction between saliva and tongue is weak and thus the droplets interacting with saliva are washed away easily.

Furthermore, Figure 5.6 shows that with emulsions more sensitive towards coalescence more fat is retained at the tongue surface than with less sensitive emulsions. This means that the *ex vivo* Raman measurements using a model surface (pig's tongue) confirm the *in vivo* findings of Figure 5.1.

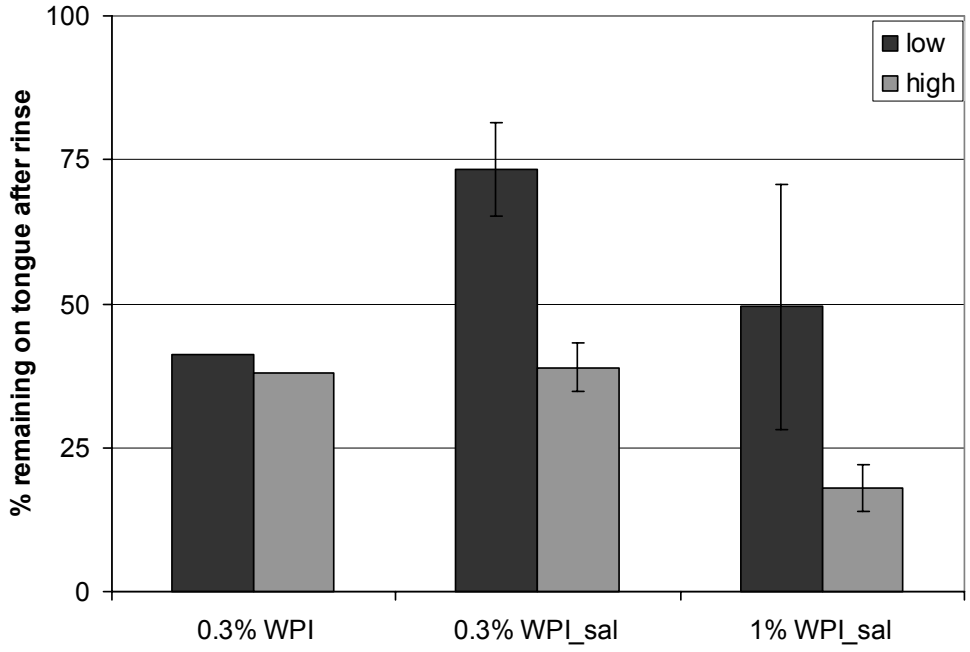

Figure 5.6: Raman shift analyses at 1658 cm^{-1} as indicator of fat retention at Pig's tongue after rinsing at low flow and high flow relative to the no rinsing situation. Emulsions differing in stability (1% WPI vs. 0.3% WPI) and saliva coated (_sal) and non-coated surfaces are used.

Emulsion droplet detection

Raman spectroscopy, while very useful in detecting the presence of fat detection, does not provide information on the form in which fat is present on the tongue. Is it present as a thin layer of spread emulsion droplets or as adhered emulsion droplets? CSLM analysis on *ex vivo* fat retention is very suitable for detecting adhered intact emulsion droplets. Figure 5.7 shows that saliva initially increases adhesion of emulsion droplets to the surface. Rinsing the pig's tongue at a low flow reduces the number of visible emulsion droplets, drastically, confirming our results of Figure 5.6.

Independent of the presence of saliva, more emulsion droplets are visible for a stable (1% WPI) emulsion when brought into contact with pig's tongue than for an unstable (0.3% WPI) emulsion (Figure 5.8). This is in contrast with the results obtained using Raman Spectroscopy (Figure 5.6) and the *in vivo* measurements (Figure 5.1). Most likely, the unstable emulsion (0.3% WPI) has a stronger tendency to spread on the surface than the stable emulsion. The confocal microscope has a spatial resolution of only around 0.5 μm and thus an emulsion droplet of 1 μm, which has spread on the surface forming a thin layer, can no longer be detected using CSLM.

5.4 Discussion

Emulsions that are more sensitive towards coalescence give rise to a lower *in vivo* oral perceived friction (Chapter 3) as well as a lower *ex vivo* measured friction (Chapter 3 & 4). As argued *and* measured before the conditions in the mouth (surface roughness, deformability, speed) and the biological function of the tongue in food handling are such that we expect boundary and/or mixed lubrication in the mouth. This means that (in-mouth) friction is mainly determined by interaction of the surface with the lubricant. Our observations reported in Chapter 3 and 4 would therefore imply an enhanced fat retention in the mouth for those emulsions that are more sensitive to coalescence. Indeed, our *in vivo* and *ex vivo* results on fat retention obtained in the present study confirm this hypothesis.

To our knowledge, almost no study focussed on oral retention phenomena. In the one study we found De Jongh and co-workers[15] investigated the influence of different thickening agents (added to emulsion-based dressings) on the kinetics of oil retention.

Unfortunately, their findings are not relevant for our study, which is focussed on understanding the relation between emulsion stability and oral fat retention.

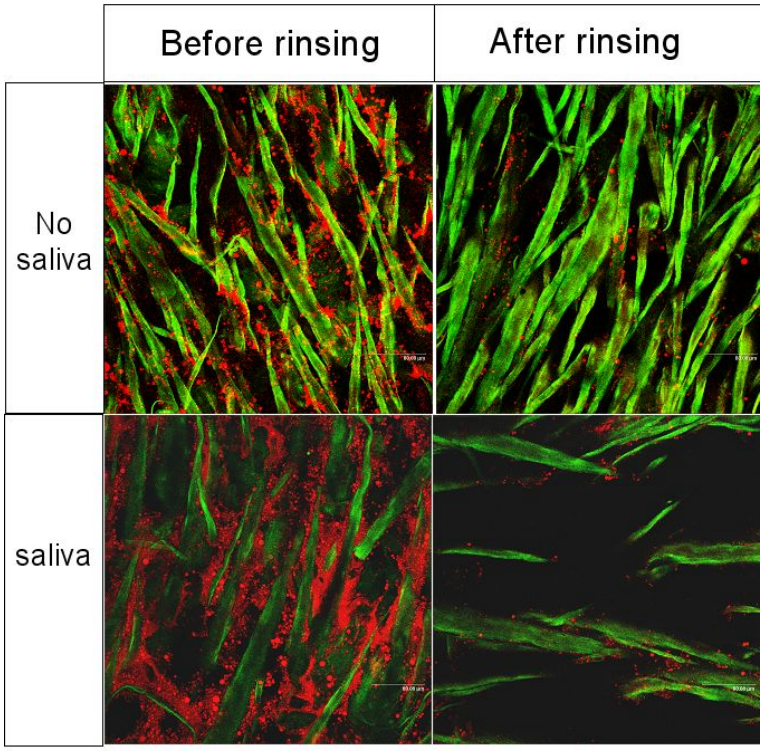

Figure 5.7: CSLM images of Pig's tongue in contact with 1% WPI 10% emulsion in the presence and absence of saliva and before and after rinsing at low flow with water. Image size 500 x 500 μm. Fat is coloured red and tongue papillae green.

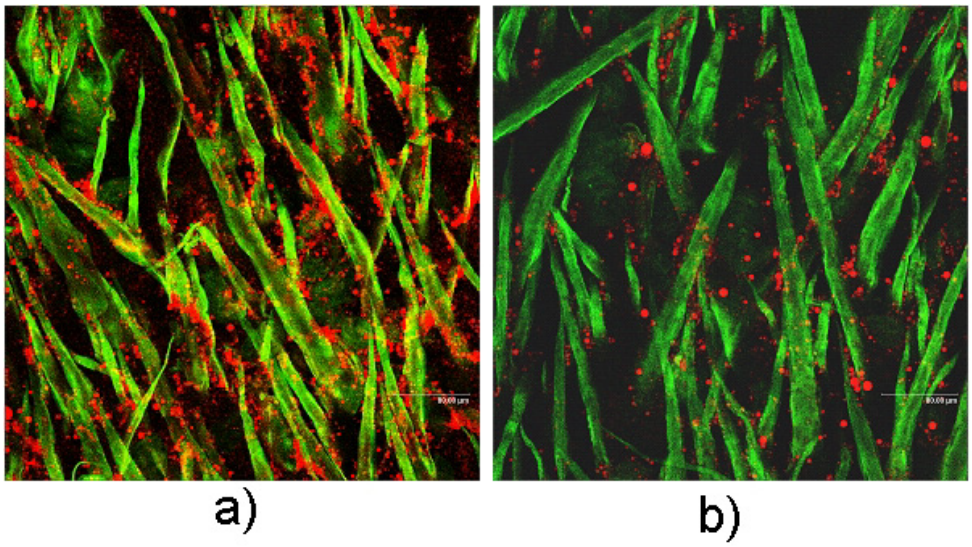

Figure 5.8: CSLM images of Pig's tongue in contact with a) 1% WPI 10% emulsion and b) 0.3% WPI 10% emulsion in the absence of saliva. Image size 500 x 500 μm

Besides decreasing emulsion stability, increasing the fat content of the emulsions resulted in an increase in total amount of retained fat. However, the relative fat retention (grams retained fat per grams of emulsified oil intake) decreased. This suggests that the accessible area on the tongue surface, and/or the time available for the droplets to adhere is limited. Note that the absolute amount of retained fat after consuming an unstable 40 wt% oil emulsion is almost equal to that after processing pure sunflower oil, but only four 4 times as much as that after processing the unstable 7 wt% oil emulsion.

From earlier observations we know that, in absence of saliva, the fat content (range 1-40 wt% oil) of the lubricating emulsions has hardly any influence on the friction (see Chapter 4). However, there is a clear difference in friction between lubrication with no oil present in the lubricant, and little oil (1%) (Chapter 4). Together with our observation that the area for fat retention on the tongue is limited, this implies that there is a minimum amount of oil (presumably less than 1 wt%) necessary to lower the friction.

However, when saliva is present the fat content does have an influence on oral perceived friction. As we argued before in Chapter 4, retained fat might form a reservoir, which counteracts the natural "cleaning" exerted by saliva, thus maintaining the low oral friction situation for a longer time. Indeed, the *in vivo* experiments show that saliva is able to quickly reduce the amount of retained fat (Figure 5.3). Moreover, saliva has been found to be very surface active [6] and to be able to induce flocculation of emulsion droplets through electrostatic and/or depletion interaction (depending on the conditions) [10]. Presumably, through creating large flocs and through adsorption of the surface-active (mucine) saliva components to the oil/water interface, saliva is capable of removing emulsion droplets and/or fat from the oral surface.

Does saliva also influence fat retention at the tongue surface? Raman spectroscopy and CSLM experiments show an initial increase in retention of fat due to saliva. However, upon rinsing, the amount of retained fat is reduced drastically. After rinsing, the difference in amount of retained fat between a saliva-covered and a saliva-free tongue almost disappears. This may indicate that saliva has only a minor contribution to retention of fat. Furthermore, saliva does not change the difference in fat retention between stable and unstable emulsions. In other words, independent of the presence of saliva, more fat is retained at the tongue surface with unstable than with stable emulsions.

To understand the effect of emulsion stability on (oral) fat retention, let us consider 3 contributions to oral retention: 1) physical entrapment of the emulsion between the papillae on the tongue 2) adhesion of emulsion droplets at the hydrophobic tongue surface 3) spreading of emulsion droplets subsequent to adhesion of the emulsion droplets.

Physical entrapment is defined here as liquid remaining between the papillae after expectorating the emulsion. Adams and co-workers [16] found for polymer solutions differing in viscosity (between 0.02 and 200 Pa.s) that more polymer remains on the tongue at higher viscosities. The viscosity of the emulsions does not depend on the protein load at the interface and, thus, no difference in physical entrapment is expected between stable and unstable emulsions.

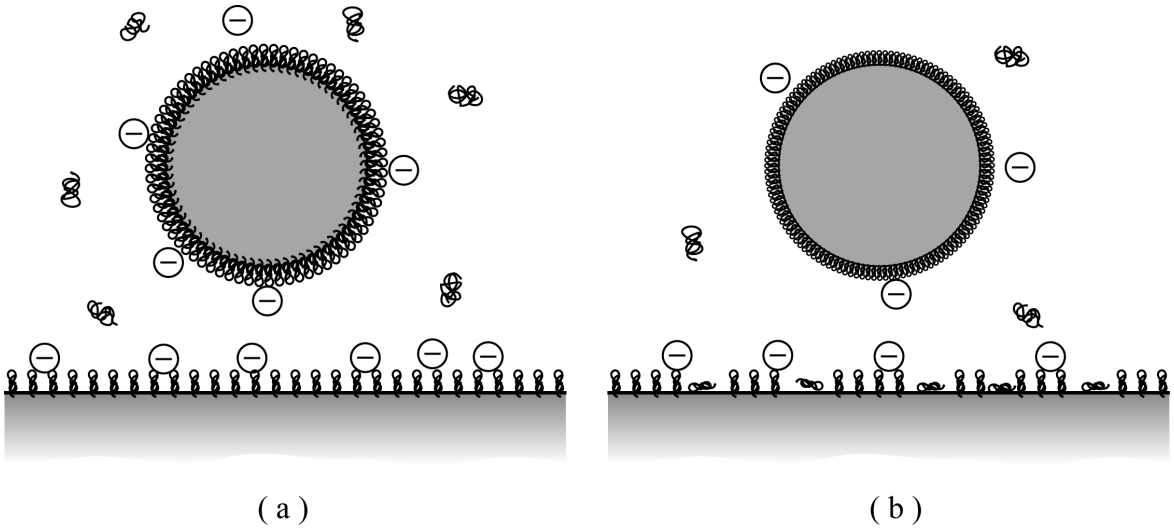

Figure 5.9: Schematic representation of high protein load emulsions being lower in adhesion energy due to an increased electrostatic and steric repulsion: a) a stable droplet with a high protein load b) an unstable droplet with a low protein load approach a hydrophobic surface.

In contrast to physical entrapment, differences in adhesion of droplets could possibly explain the higher oral fat retention of less stable droplets. To analyse this further we first consider colloidal forces between the tongue and the emulsion droplets at long separation distance (>10nm) for which the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory provides adequate predictions. What are the possible differences in electrostatic and Van der Waals interaction forces between the stable, protein-rich and the unstable, protein-poor emulsion droplets? Figure 5.9 shows a schematic representation of the two

types of emulsion droplets approaching a hydrophobic solid surface covered with protein. At the pH used here, the emulsion droplets have in both cases a net negative surface charge, but due to varying protein concentration during emulsification, the surface load (adsorbed amount) is less for the unstable (protein-poor) emulsion droplets than for the stable (protein-rich) droplets [17; 18]. Literature data indicate that the surface load for the unstable emulsion is ≈2.5 - 4.5 mg m^{-2} (depending on the conditions) and for the stable emulsion ≈6.5 - 7.2 mg m^{-2} [18]. This will result in a difference in amount of charges on the droplet surface, as illustrated in Figure 5.9. However, we estimated (from compositional data on BiPro WPI [19]) that the ionic strength in the continuous phase of our emulsions lies around 10mM, which corresponds to a screening length of around 3 nm. This shows that at large separation (>10nm) there is for both emulsions hardly any contribution of electrostatic interaction on the total interaction potential.

The difference in Van der Waals interaction forces for the two types of droplets can be estimated from classical expressions [20]. At close separation, dielectric properties of the adsorbed layer are reported to have a significant impact on droplet/solid surface total interaction potential [20]. However, at large separation distances the total interaction potential mainly depends on only the differences in dielectric properties between oil, water and tongue, which are the same for stable and unstable emulsions. In short, at long separation distances there is no difference in the interaction potential between low protein load and high protein load emulsion droplets approaching a solid surface, indicating that the differences in adhesion between the two types of emulsion droplets is mainly due to differences in interaction potential at short separation distances.

At short separation distances (< 10nm), besides Van der Waals and electrostatic interactions, more forces come into play. Without very precise molecular models of the adsorbed layer, it is extremely difficult to predict interactions and to determine which interactions are dominant. At best, we can try to identify interaction forces that are likely to differ as function of protein load on the droplet (emulsion stability).

First, the short-range steric interaction forces may play a role. For unstable (protein-poor) emulsion droplets the adsorbed amount of protein is lower, and therefore the protein can unfold more at the o/w interface, resulting in a thinner adsorbed layer than with the stable (protein-rich) emulsion droplets. Moreover, at higher protein

concentrations also formation of multi-layers has been reported which could result in even thicker interfacial layers [18]. Clearly, the protein-poor droplets have a decreased steric repulsion in comparison to the protein-rich droplets [21]. Short-range hydrophobic interaction forces are also expected to be stronger for protein-poor droplets (unstable) than for protein-rich droplets (stable) since more protein is expected to unfold at the interface and then more hydrophobic groups are exposed to water. In other words, the driving force to reduce the amount of polar/apolar interfacial area is stronger for protein-poor than for protein-rich droplets. Finally, the electrostatic repulsion forces are less for protein-poor (unstable) emulsions than for protein-rich (stable) emulsions due to, as explained earlier, less surface charge on the unstable droplet as on the stable droplet.

In summary, hydrophobic, electrostatic and steric interactions vary with interfacial protein load at short separation distances. It has already been reported that the impact of these interactions on adhesion highly depends on the magnitude of the total interaction potential and that for food emulsions these interactions can have significant impact [18; 21]. In conclusion, protein-poor emulsion droplets are likely to be more prone to adhere than protein-rich droplets; this conclusion is consistent with the results of our experiments.

The last contribution to oral retention we consider is spreading of emulsion droplets, which may occur after adhesion. Spreading of an emulsion droplet can take place when the interfacial layer ruptures, allowing the oil phase to wet the hydrophobic surface. Figure 5.2 and 5.3 show that the retained fat layer is harder to remove of protein-poor emulsions (unstable) than of protein-rich emulsions (stable), suggesting that in the former case a larger percentage of the droplets has spread on the surface. Spreading of oil makes the retained fat layer more stable against rinsing since only strong hydrophobic forces between the hydrophobic surface and the apolar oil are involved, and no repulsion. From previous work (see Chapter 3 & 4) we have indications that spreading of emulsion droplets is the main reason that emulsions are efficient in lubricating surfaces, unstable emulsions being more effective. Both the *in vivo* and the Raman measurements show that the oral fat retention increased when stability of the emulsion droplets decreased. Again, the difference in protein load (in this case through difference in stability of the interfacial layer against rupture) determines the vulnerability of emulsion droplets against spreading.

In summary, emulsion droplets stabilised by low protein loads adhere more strongly to hydrophobic surfaces such as the tongue than high protein stabilised emulsion droplets, and when they do adhere they are more prone to spread.

5.5 Conclusion

Emulsions stabilised by less protein and therefore more sensitive towards coalescence, are more prone to not only adhere to the tongue surface, but also spread on the surface. In the light of earlier tribological experiments in combination with sensory analyses, our results show that the sensitivity of an emulsion droplet to first adhere to the tongue surface and subsequently spread on this surface determines in-mouth friction and therefore perception of fat. Saliva also has an effect on retention of fat to the oral surface, but its exact role needs further research. Given the observed fast decrease of fat in time on the oral surface, we conclude that saliva is of importance in removing oil from the hydrophobic tongue surface. Unstable emulsion droplets adhere stronger and spread more easily on the oral surface than the stable emulsion droplets. This is consistent with expectations based on the differences in the colloidal interaction forces between the droplets and the tongue surface for droplets poor in protein and droplets rich in protein.

Acknowledgement

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6

Direct observation of adhesion and spreading of emulsion droplets at solid surfaces

Abstract

Sensory perception of fat is related to orally perceived in-mouth friction. In this perspective, we investigate adhesion and spreading of emulsion droplets on solid surfaces and connect it to the ability of food emulsions to lower friction. Furthermore, we study what the contribution is of the separate colloidal forces on droplet adhesion. Briefly, also the effect of saliva on adhesion and spreading is investigated.

Using a flow cell in combination with light microscopy and video imaging allowed to clearly distinguish between adhered and spread emulsion droplets. The capability to make this distinction between adhesion and spreading experimentally is new and provided us with the insight that occurrence of spreading is essential for lowering friction. Mainly electrostatic, steric and hydrophobic interactions of the droplets with solid surfaces are found to determine adhesion and subsequent spreading of emulsion droplets. This was investigated by varying the adsorbed amount of protein, the ionic strength of the emulsions as well as the hydrophobicity of the solid surface. Especially the hydrophobic interaction between droplet and surface is shown to be crucial for droplet adhesion and spreading. Saliva is of minor importance for adhesion and spreading. This work gives insight in the way emulsion droplets interact with solid surfaces and the type of colloidal interactions that play a role. The information it provides can be used to develop emulsions that are reasonable stable during the shelf life of the product, but do spread on oral surfaces, thus lowering friction and enhancing fat perception.

D.M Dresselhuis, G.A. van Aken, E.H.A. de Hoog, M.A. Cohen Stuart

6.1 Introduction

The ability of oil-in-water emulsions to act as a lubricant is currently of great interest in the personal care industry, in food science and in the food industry. Traditionally, emulsion lubricants were mainly of interest in manufacturing processes, especially metal working operations [1]. The reasons for studying emulsions as a lubricant vary between the different research areas. However, in all cases the mechanism by which emulsions lower the friction is still barely understood [1; 2]. An important reason why food industry and science are interested in emulsion lubrication lies in the role lubrication plays in fat perception ([3-7] and Chapter 3). Understanding in-mouth emulsion lubrication would provide the food industry with tools to enhance fat perception of low fat food emulsions.

In earlier work, we made some significant progress in understanding the mechanisms through which emulsions can lower friction. We found that an increased retention of fat (originating from food emulsion droplets) at solid surfaces, such as the tongue, is related to a reduced friction (Chapter 4 & 5). More importantly, this work gave indications that it is not adhesion of emulsion droplets as such which causes this increased fat retention *and* which lowers the friction, but rather the subsequent spreading of the droplets (Chapter 4 & 5).

The reasons why protein-stabilised emulsion droplets adhere and spread at solid surfaces are poorly understood. There is even no literature we are aware of which gives direct evidence that spreading of protein-stabilised emulsion droplets on solid surfaces actually occurs. Also suggestions in literature that spreading of surfactant-stabilised emulsion droplets occur, are sparse [8; 9]. The use of hydrophobic membranes to facilitate coalescence of surfactant-stabilised droplets in waste water treatment is widely accepted. This suggests that spreading is of importance in separating oil from water. However also here, the mechanism of coalescence of droplets in the pores also remains largely unclear [10-12]. The reason lies probably in experimental difficulties with studying emulsion droplet adhesion. Methods commonly used to study adsorption of molecules to solid surfaces, like reflectometry and evanescent wave spectroscopy, are not suitable due to the relatively large size of food emulsion droplets.

Spreading of emulsion droplets can occur after adhesion has taken place, through rupture of the interfacial layer (often a protein layer in case of a food emulsion), allowing the apolar oil to wet hydrophobic surfaces (such as the tongue (see Chapter 2)). We have found that emulsion droplets stabilised by low amounts of protein (indicated as 'protein-poor' emulsion droplets) are more prone to adhere and spread on oral surfaces than more stable emulsion droplets stabilised with high amounts of protein (indicated as 'protein-rich' emulsion droplets) (see Chapter 5). The presence or absence of saliva did not affect this dependency on emulsifier load (Chapter 5). In connection to this, we should mention that Cambiella *et al.* [1] and Dubey *et al.* [2] also identified a relation between emulsifier load and friction in their studies focussing on metal lubrication.

The reason why protein-poor emulsion droplets adhere more to hydrophobic surfaces than protein-rich droplets was explained by considering the various colloidal forces involved (Chapter 5). We concluded that protein-poor droplets are expected to adhere better at solid surfaces than protein-rich emulsion droplets due to a decreased electrostatic, and steric repulsion by the surface at short separation distances (Chapter 5), allowing hydrophobic attraction to take over.

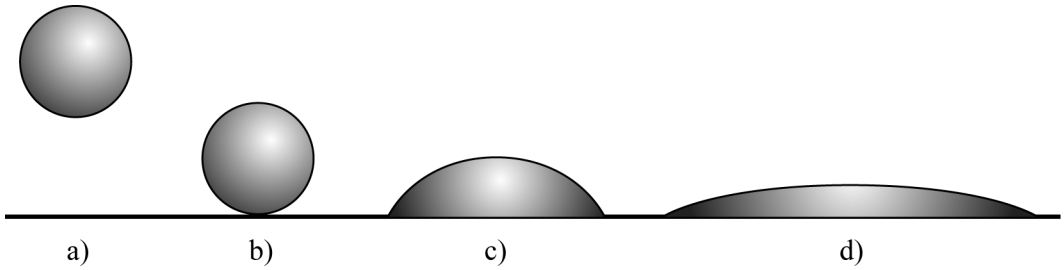

Figure 6.1: Schematic representation of different appearances of emulsion droplets close to a solid surface a) non-adhered droplet b) adhered droplet c) spread droplet, large contact angle d) spread droplet, small contact angle.

In the present work, we study in more detail the influence of these various colloidal forces on droplet adhesion and spreading. For this purpose, we introduce a method to observe droplet adhesion and spreading, which is not limited by the size of the emulsion droplets. This method combines a flow cell with light microscopy and a CCD camera. With this set-up we aim to distinguish three different emulsion droplet conditions close to the solid surface: a) non-adhered droplet b) adhered droplet and c & d) spread droplet (see Figure 6.1). Using this method we will study whether indeed more spreading occurs

with the protein-poor than with the protein-rich emulsions. Furthermore, we investigated the role of electrostatic and hydrophobic interactions by varying the ionic strength of the emulsions and the hydrophobicity of the solid surface. Also, the effect of presence of salivary proteins on emulsion droplets adhesion and spreading was studied briefly.

6.2 Material & methods

6.2.1 Emulsion preparation and characterisation

O/W emulsions containing 40 wt% oil were prepared by mixing oil with protein solutions using an ultraturrax (T25 basic, IKA, Staufen, Germany, at 17500rpm) for 4 minutes at room temperature. The emulsions were stabilised by either 1 wt% or 0.3 wt% Whey Protein Isolate (WPI; BiPro, Davisco, USA). Sunflower oil (SF; fully winterized, η= 60mPa.s, kindly provided by Cargill Amsterdam, The Netherlands) was used as the oil phase. The 40 wt% emulsions were diluted prior to each experiment with demineralised water or with a buffer solution to obtain a 0.5 wt% SF oil emulsion. The NaCl (Merck, Darmstadt, Germany) concentration in the continuous phase ranged from 0 – 150 mM NaCl and pH of the buffer was pH 6 (citric acid/ NaOH, 0.005 M, Merck, Darmstadt, Germany).

Table 6.1: Average particle size of emulsion differing in protein load

	D[3.2] μm	D[4.3] μm
0.3 wt% WPI 40 wt% SF	12.3	23.0
1 wt% WPI 40 wt% SF	13.4	23.0

The 1 wt% WPI stabilised emulsions were previously shown to be stable against coalescence in the mouth, whereas the 0.3 wt% WPI stabilised emulsions were shown to be less stable against coalescence (Chapter 3) under the same conditions. Note that due to varying protein concentration during emulsification, the surface load will be different [13; 14]. The average particle size of the emulsions was determined by light scattering (Mastersizer 2000, Malvern, Worchestershire, UK) (see table 6.1) and remained stable during the experiments.

6.2.2 Solid surface preparation and characterisation

Glass microscope slides (Menzel Gläser, Braunschweig, Germany) were used as solid surfaces in the adherence and spreading experiments. They were cleaned thoroughly with a mixture of 2/3 concentrated sulphuric acid and 1/3 hydrogen peroxide. The clean slides were rinsed with MilliQ water, dried and oxidised by oxygen-plasma-treatment for 2 minutes in a plasma-cleaner (Harrick PDC-32 G, Anadis instruments, Malden, The Netherlands) and stored in demineralised water (glass is hydrophilic). Hydrophobic glass slides were obtained by placing the cleaned slides in an desicator containing a saturated hexamethyldisilazan (HDMS, Sigma-Aldrich, Germany) vapour for minimum 12 hours. The air in the desicator was replaced with nitrogen. Measurements of the water/glass/air contact angle confirmed the hydrophobicity of the surfaces. Simple streaming potential measurements showed that both the hydrophobic and hydrophilic slides were negatively charged, the latter being most charged. The hydrophilic (Phi) and hydrophobic (Pho) slides were coated with saliva by covering the whole slide with 2 ml unstimulated, fresh, non-centrifuged saliva of one person for 45 minutes (evaporation was largely prevented). Unstimulated saliva is rich in mucins (large glycoproteins with sialic acid side chains [15], and references there in). The saliva-covered surfaces were placed in the flow cell without removing the excess saliva. For the contact angle measurements the excess saliva was washed away at low flow and the slides were dried at room temperature against air.

6.2.3 Flow cell measurements

The flow cell set-up consisted of a pump (Microspump serie 200, Axel Johnson International, Almere, The Netherlands) generating a non-pulsating flow, a flow chamber and a light microscope (magnification 100x, Olympus BX60, Zoeterwoude, The Netherlands). In the flow chamber (Figure 6.2) the silica plate (B) and the replaceable test surface (A) are 1 mm apart and the flow is laminar. The diluted emulsion was injected in the flow cell set-up, which contained either demineralised water or the appropriate buffer solution. The emulsion was first allowed to adhere for 5 minutes (no flow) after which the fluid in the set-up was circulated for 10 minutes at low flow (around 20ml/min). Note that emulsion droplets cream, which might increase the chance of adhesion of droplets. Subsequently, the system was rinsed at 10ml/min with either demineralised water or

buffer solution for at least 10 minutes after which only adhered or spread emulsion droplets could be observed. Images were taken with a CCD camera (Olympus DP70, Zoeterwoude, The Netherlands). Before each experiment a new glass plate was used (Figure 6.2, A) and the system was cleaned and rinsed thoroughly to remove protein and fat using enzymatic soap (Tergazyme, Alconox, USA) and (demineralised) water, following each time the same procedure. Since the emulsions were highly diluted, hardly any protein was present in the continuous phase and therefore also hardly any adsorption of free protein on the solid surface is expected. The experiments were performed in duplo.

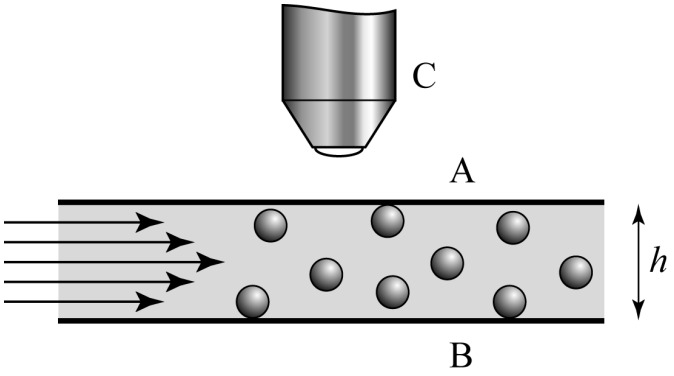

Figure 6.2: Schematic representation of the flow cell set up with laminar flow at variable flow rates with A) replaceable glass plate B) silica plate C) light microscope with CCD camera h) 1 mm distance between plates.

6.3 Results and Discussion

6.3.1 Distinction between adherence and spreading of emulsion droplets

Light microscopy is widely used to study emulsion droplet characteristics such as degree of aggregation and droplet size. In this paragraph we use light microscopy in combination with a flow cell and a CCD camera to study emulsion droplet adhesion and spreading at a solid surface.

Figure 6.3 shows the different droplet appearances observed in our combined flow cell/microscopy experiments. Image 6.3a shows a perfectly spherical droplet, which could be distinguished from the background through the presence of its surrounding dark edge. This is the appearance of an emulsion droplet as observed with light microscopy in numerous studies. Applying a flow, and using a CCD-camera reveals that an adhered emulsion droplet had the same appearance as a non-adhered droplet. Note that in figure

6.3 only adhered and spread emulsion droplets are observed since the non-adhered droplets are rinsed away (see §6.2.3). This is also the case for all the figures shown in the next paragraphs.

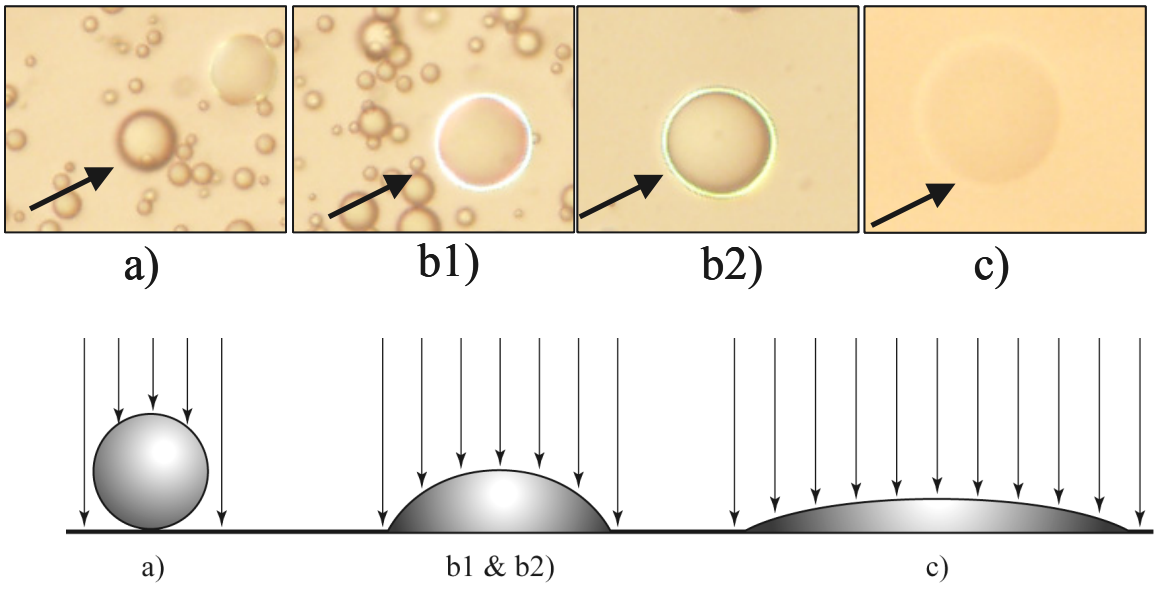

Figure 6.3: Different microscopic appearances of emulsion droplets in contact with a hydrophobic solid surface and the schematic expected side view; a) adhered droplet, b1) spread droplet with relative large contact angle, b2) spread droplet with different contact angle, c) spread emulsion droplet with a relative small contact angle.

Using the CCD-camera allowed us to observe spreading of emulsion droplets (image 6.3b&c), which occurred either almost instantaneously or after the droplets had been adhered at the solid surface for a certain time. Spreading could be clearly discerned from adhesion by the observed sudden change in diameter of the droplet, as well as the change in colour of the edges of the droplet: spread droplets are surrounded by a bright ring. Figure 6.3 shows that using a flow cell in combination with light microscopy and CCD camera enables to distinguish between non-adhered, adhered and spread emulsion droplets.

Besides this distinction, Figure 6.3 also shows three different appearances of spread emulsion droplets. Images 6.3b1 and 6.3b2 show that reflected light of the edges of the two droplets differs in spectral characteristics. This differs from the non-reflecting black edge of the spherical shaped adhered droplets (image 6.3a). The coloured edges of the droplets are the result of thin film interference phenomena, which are visible when the

film thickness is on the order of the wavelength of visible light. Differences in contact angle result in differences in film thickness at the edges of the droplets (see schematic side view Figure 6.3). Depending on this film thickness, the angle of incidence, the refractive indices of the two media and the order of the phase shift, constructive or destructive interference can occur [16]. With visible light these interference phenomena can be observed as a change in colour of the reflected light, like seen in figure 6.3. Most likely, the curvature of the interface also influences interference phenomena.

When the contact angle decreases further (image 6.3c), the visible interference phenomena disappear. This is due to the change in film thickness and curvature. For a very thin ’pancake’-shaped droplet the interference effects become too small to produce visible colours. In other words, the observed colour of the edge of the droplet indicates that the droplets are spread at the surface with a certain contact angle. Further analysis of the relation between the contact angle and interference phenomena was outside the scope of this study.

Direct observations of emulsion droplet adhesion at solid surfaces are sparsely described in literature and with the observation of spreading of emulsion droplets on solid surfaces we enter a new research area. In an experiment similar to ours, Essafi *et al.* [17] determined adhesion of emulsion droplets through video-counting of the number of droplets which were stuck at the solid surface. Moreover, Essafi and co-workers, like Poulin and Bibette [18], claim that the amount of adhesive energy of the droplets can be determined by measuring the contact area between droplet and solid wall. Since the droplets used in our experiments were too small in size (factor 2-6 smaller than the ones of Poulin *et al.* and Essafi *et al.*) the resolution of the light microscope was insufficient to determine the adhesive contact area using this method. Neither Essafi *et al.*, nor Poulin & Bibette *et al.* nor other authors we are aware of report about the direct observation of spreading of emulsion droplets on solid surfaces.

6.3.2 Influence of amount of salt and emulsifier

Previous work showed that altering the characteristics of the interfacial layer greatly influences adhesion, and possibly spreading of emulsion droplets. Here, we studied the impact of two interfacial layer characteristics: the amount of interfacial protein

(protein-rich vs. protein-poor), and the amount of charge. We first discuss the influence of protein load.

Figure 6.4 shows that on hydrophobic surfaces the unstable, protein-poor emulsion droplets adhere and spread more than protein-rich droplets. This direct observation supports earlier (indirect) indications, that lowering of the amount of interfacial protein not only increases adhesion, but also spreading of emulsion droplets on (hydrophobic) oral surfaces (Chapter 4 & 5). In this previous work an enhanced *in vivo* and *ex vivo* fat retention was found upon lowering the emulsifier load. The observed enhanced spreading tendency of protein-poor emulsion droplets (Figure 6.4) might be the reason that these emulsions were found to be more efficient as lubricant than protein-rich emulsions (Chapter 4 & 5). Since sensory fat perception is related to lubrication, these findings indicate that the ability of emulsions to spread on the surface is essential in mouth-feel related fat perception. It is therefore of interest to find ways to increase spreading of emulsion droplets on hydrophobic surfaces, without decreasing the stability of the droplets in the bulk.

There are several ways to increase spreading of droplets. Since prior to spreading, the droplet should make contact with the surface, in other words adhere to the surface, one way to increase spreading is to increase the number of adhered droplets. Assuming that the probability that the interfacial layer of the adhered droplets ruptures is constant, more adhesion will increase the number of droplets that spread. Another way to increase the amount of droplets that spread is to increase the susceptibility of the interfacial layer to rupture. Once a hole is formed, the balance between the interfacial tensions at the oil/water/solid phase boundary predicts to what extent oil wets the surface [19].

$$S = \gamma_{os} - (\gamma_{ws} + \gamma_{ow}) \quad \text{(eq 6.1)}$$

Here, γ_{os} is the interfacial tension between oil and glass (here hydrophobic), γ_{sw} between (hydrophobic) glass and water, and γ_{ow} between oil and water. For $S > 0$, oil spreads completely (zero contact angle), whereas for negative values of S, a finite contact angle results in: $\cos \theta = 1 + S$. In Figure 6.4 we observe droplets with very small contact angles. Apparently, the spreading coefficient, S, is close to zero, or perhaps even positive. This means that the sum of the interfacial tensions of water/hydrophobic glass and

oil/water is comparable to that of oil/hydrophobic glass, resulting in oil spreading at the hydrophobised glass, wetting the solid surface.

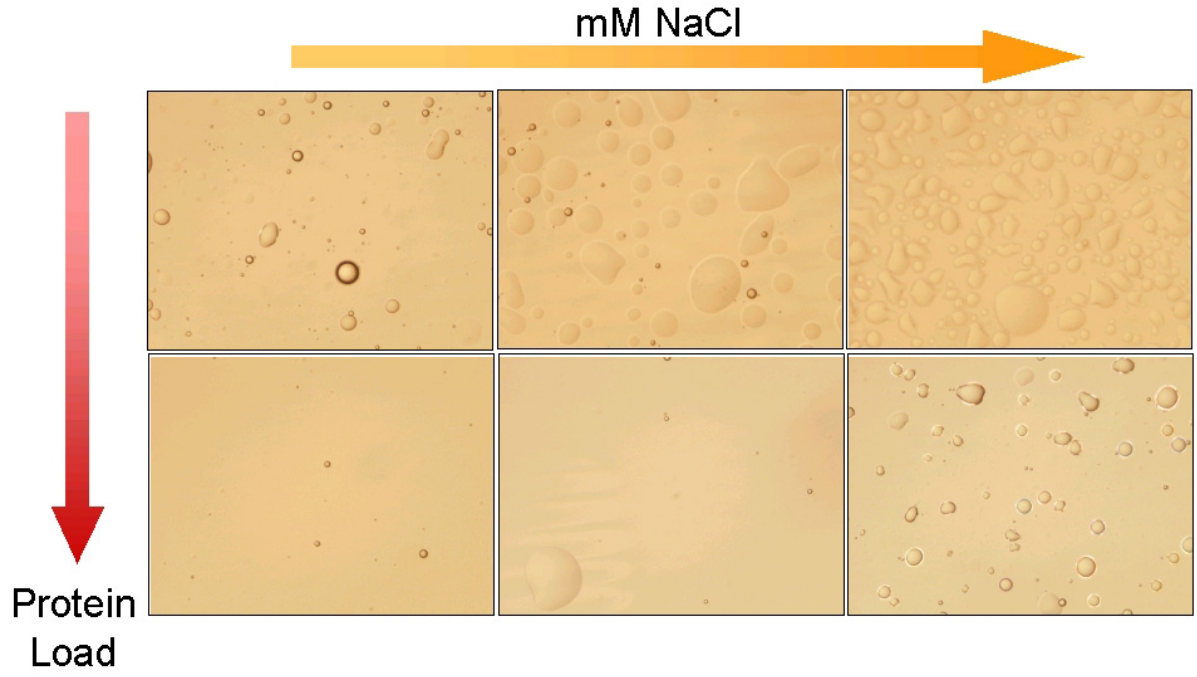

Figure 6.4: Adherence and spreading of emulsion droplets as function of increasing salt concentration (0-10-50 mM NaCl) and increasing stability of emulsion droplets (0.3% WPI vs. 1% WPI) on hydrophobic glass.

It is expected that one way to increase the amount of adhered emulsion droplets and thus increase spreading is to *decrease* electrostatic repulsion between in this case the negatively charged surface and the negatively charged emulsion droplets (Chapter 5). Indeed, Figure 6.4 shows that decreasing the electrostatic repulsion by increasing the ionic strength of the continuous phase enhances the susceptibility of both protein-poor and protein-rich emulsion droplets for adhesion and spreading. This is in agreement with Poulin & Bibette [18] and Essafi and co-workers [17] who also found this relation between ionic strength and adhesion of anionic (in their case) surfactant stabilised emulsions. In a different type of study, using ellipsometry, Malmsten and co-workers [9] also found an increase in adhered amount of surfactant-stabilised emulsion droplets upon increasing the ionic strength. However, the authors urged to be careful when drawing conclusion from this ellipsometry data on the form in which droplets were present in the adhered layer. The size of the used droplets was in this study comparable with the wavelength of visible light, and the formed film was inhomogeneous, which both made

interpretation on the structure of the adhered layer difficult. Note that no aggregation of emulsion droplets in the emulsions of Figure 6.4 was observed as function of the high salt concentration, which indicates that droplet-droplet interaction is still sufficiently repulsive. The effect of adding salt on adhesion and spreading was less pronounced for the protein-rich emulsion droplets. This could be due to the relatively higher surface charge density on the protein-rich emulsion droplets (Chapter 5). The electrostatic repulsion depends on the surface charge density (σ), the surface potential (Ψ_s) and the Debye screening length (κ^{-1}) [20]. Increasing the ionic strength of the 1:1 electrolyte NaCl from 50 mM until 150 mM decreases κ^{-1} from 1.4 nm to 0.78 nm (at low surface potentials < 25 mV, and T = 298 K [20]). This explains the observed increase (Figure 6.4) in adhesion and spreading of droplets as function of ionic strength. However, at fixed ionic strength an increase in charge density, results in increase in surface potential, which increases the electrostatic repulsion [21; 22]. This implies that, differences in charge density between protein-poor and protein-rich stabilised emulsion droplets will result in differences in electrostatic repulsion. This could explain the difference in observed adhesion and spreading of protein-poor vs. protein-rich emulsion droplets (Figure 6.4). The significance of the effect depends on the balance between repulsive and attractive interaction forces.

A possible second reason for this difference in effect of adding salt on emulsion droplets adhesion as function of protein load is the higher *steric* repulsion for the protein-rich emulsion droplets in comparison to the protein-poor. Due to higher protein concentrations during emulsification, the proteins have less time to unfold at the oil-water interface than at lower concentrations of proteins. This results in higher amounts of more compact protein present in the interface [14] and a thicker adsorbed layer. In other words, decreasing the electrostatic repulsion and possibly steric repulsion between droplet and surface has been shown to indeed increase the probability of spreading of emulsion droplets.

Another way to increase spreading is to increase the susceptibility of the interfacial layer for rupture. Upon increasing the protein load (and thus increasing the thickness) at the interface not only the steric repulsion is influenced, but thicker interfacial layers are also known to provide extra mechanical stability against rupture [22]. If a protein layer has internal cohesion (which is very likely) creating a hole will cost more free energy if

the layer is thicker; the probability of rupture will decay with increasing hole energy.

6.3.3 The influence of hydrophobicity of the solid surface

Besides steric and electrostatic (see §6.3.2) also hydrophobic interactions are expected to play a role in emulsion droplet adhesion and consequently spreading at solid surfaces. Due to the adhesion of droplets the polar/apolar interfacial area of the hydrophobic surface with water is reduced. Therefore, more droplets are expected to adhere and spread at a hydrophobic surface than at a hydrophilic one.

Figure 6.5 confirms this expectation and shows that emulsion droplets hardly adhere and definitely do not spread at the hydrophilic surface in comparison to the hydrophobic one. Even increasing the salt concentration up to 150 mM does not increase the amount of adhered droplets on the hydrophilic surface.

The absence of any adhered droplet at such high ionic strength suggests that either the electrostatic repulsion between the negatively charged hydrophilic surface and the negatively charged emulsion droplets is extremely high, or the attractive forces (such as hydrophobic attraction) between the droplets and the solid surface are lower for the hydrophilic surface than for the hydrophobic surface.

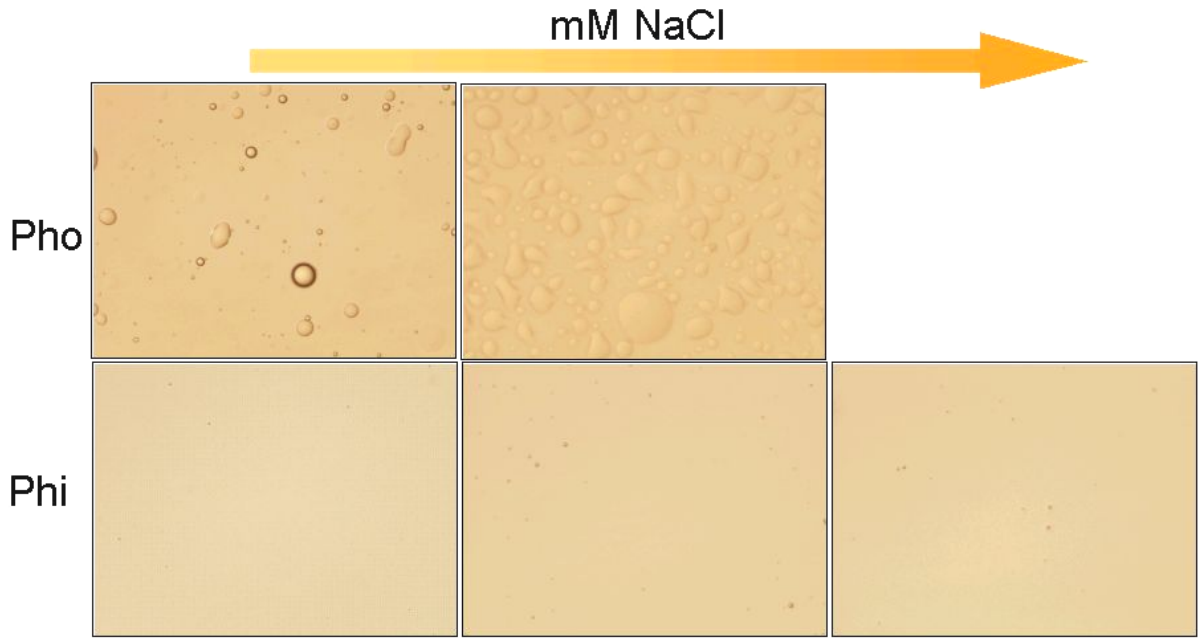

Figure 6.5: Adherence and spreading of unstable emulsion droplets (0.3% WPI) as function of increasing salt concentration (0-50-150 mM NaCl) at hydrophobic (Pho) and hydrophilic (Phi) glass.

Let us first consider the option that the electrostatic repulsion is, even at high ionic strength, higher between the hydrophilic surface and an emulsion droplet, than between the same droplet and the hydrophobic surface. At relative high ionic strength (150mM) the Debye screening length is very small (~0.8 nm), and almost a factor 4 smaller than the screening length at a relatively low ionic strength of 10 mM. The charge density of the hydrophilic surface is higher than that of the hydrophobic surface. Both charge density and Debye screening length influence electrostatic repulsion. However, the difference in charge density is far less than a factor 2. Therefore, we expect that the electrostatic repulsion between an emulsion droplet and the hydrophilic surface in presence of 150 mM salt is smaller, but at least equal to the repulsion between the same droplet and the hydrophobic surface in presence of 10 mM salt. This implies that the difference in amount of adhered droplets in Figure 6.5 between the situation hydrophobic surface 10 mM and hydrophilic surface 150 mM can not be due to the electrostatic repulsion. This suggests that it is due to a reduced (hydrophobic) attraction and not due to an increased electrostatic repulsion that fewer droplets adhere at the hydrophilic than at the hydrophobic surface.

Another explanation, which includes consideration of the influence of attractive forces on adhesion is given by Poulin & Bibette [18]. They also find no adhesion of negatively charged emulsion droplets on hydrophilic surfaces at salt concentration ranging between 0 – 0.8 mM. Poulin & Bibette suggest that, due to the absence of an adhered surfactant layer on the hydrophilic surface, no attractive interaction could occur between adhered surfactant layer on the droplet and on the solid surface. In contrast to this finding, Michalski and co-workers [23] report an increase in adhesion of protein-stabilised food emulsions on hydrophilic surfaces. They also explain their result by suggesting the existence of attractive forces between adhered layers on the droplet and on the solid surface. Differences in adsorption of proteins to hydrophilic and hydrophobic surfaces could explain that, even at very high salt concentration, the attraction forces are less than the repulsion forces. However, in our case the concentration of protein in the continuous phase is very low and thus we do not expect an adsorbed protein layer at all at the surface. Therefore, in our case the interaction between adsorbed protein layers cannot explain the absence of adhered droplets on hydrophilic surfaces.

Another attractive interaction that could explain this difference in adhesion on hydrophobic and hydrophilic surfaces is Van der Waals interaction. Van der Waals interaction can be either attractive or repulsive when there is an asymmetric interaction, such as when an oil droplet interacts via water with glass. We argued earlier that mainly interaction at short separation distances (<10 nm) determines the differences in adhesion since, at large separation, interaction forces between droplet and surface are equal for the two surfaces (Chapter 5). In contrast to this, it is suggested that at small distances protein at the oil/water interface should be considered as one of the factors determining Van der Waals interaction between emulsion droplets and surfaces [21]. Unfortunately, it is extremely difficult to estimate the dielectric properties of the adsorbed protein layer on the emulsion droplet and, therefore, to determine whether the Van der Waals interaction forces between droplet and solid surface are repulsive or attractive. The polarizability of the surface groups on the hydrophobic and hydrophilic surface differ slightly (Si-O-Si vs Si-OH [20]) and thus a small difference in Van der Waals interaction forces is anticipated. However, since it is not clear whether the forces are attractive or repulsive, it is not possible to determine if the contribution of Van der Waals force explains or rather contradicts our finding of the absence of droplet adhesion on hydrophilic surfaces. In summary, hydrophobic interaction forces (and possibly Van der Waals interaction forces) play a crucial role in adhesion of emulsion droplets. Most likely, the absence of the possibility to have hydrophobic interactions explains the absence of adhesion and spreading of droplets.

To study droplet adhesion/spreading more quantitatively (and further increase the understanding of the influence of the colloidal forces), image analyses on stained emulsion droplets should be performed. This should be combined with detailed analyses on the characteristics of the adsorbed protein layer on both the droplets, and the solid surfaces. Using stained oil enables to distinguish more accurately the oil droplet from the background, which is needed to perform digital image analysis.

6.3.4 The influence of saliva

Since we are interested in how emulsion droplet adhesion and spreading affect oral-perceived friction, we also consider the role of saliva. Figure 6.6 shows that initially

(situation after 5 min rinsing), emulsion droplets are trapped in a thick layer of unstimulated saliva. This occurs independently of the hydrophobicity of the surface. Strictly speaking, the droplets did not adhere at the solid surface itself, but rather got stuck through interaction with components of saliva. No penetration of droplets in the salivary layer or spreading of droplets on the solid surface was observed. This suggests that the droplets only interacted with the saliva layer on the solid surface and thus were unable to reach the (hydrophobic) surface. Note that in the mouth the situation is different; in the flow cell experiment not only the amount of saliva per solid surface area is higher, but also the amount of droplets per amount of saliva is lower than in the mouth.

After rinsing for some time (25 min) at very low flow rate (less than 20 ml/min) almost all the droplets are removed, even from the hydrophobic surface (see Figure 6.6). Earlier *in vivo* experiments also suggested that saliva is very efficient in removing fat and/or droplets from the hydrophobic tongue surface (Chapter 5). In other words, the results in Figure 6.6 suggest that interaction of the droplets with saliva enables removal of emulsion droplets of the surface together with the saliva. Moreover, in this experiment the salivary layer prevents droplets from reaching the surface.

The question now is, does saliva have any influence on adhesion and spreading at the surface under in-mouth conditions at all? One way it could influence adhesion (and spreading) is by changing the hydrophobicity of the surface. Indeed, Table 6.2 shows that the hydrophobicity of the surface alters when salivary proteins are allowed to adsorb on the surface. It shows that the hydrophobic surface changes from hydrophobic into hydrophilic. Note that the salivary protein layer has been dried which differs from the in-mouth situation in which salivary proteins are present in their native, hydrated form.

In a similar set-up Ranc and co-workers [24] compared a dry pig's tongue coated with stimulated saliva with an uncoated dry tongue. They also observed a decrease in hydrophobicity, but less extreme than observed by us upon coating hydrophobised glass with unstimulated saliva (Table 6.2). Possibly, the salivary coating in Table 6.2 still contained a lot of water, which would explain the dramatic decrease in observed hydrophobicity. In other words, the experiments in Table 6.2 and Figure 6.6 as such do not provide evidence that there is, or is not, a relation between saliva and droplet adhesion and spreading. However, i*n vivo* en *ex vivo* experiments performed earlier showed that,

both in presence *and* absence of saliva, protein-poor emulsion are retained more at the tongue surface than protein-rich emulsions (Chapter 5), which support the findings in Figure 6.4 (obtained in absence of saliva). This suggests that, in the mouth, saliva is *not* capable of completely preventing adherence and spreading of emulsion droplets, otherwise the difference between the two emulsions in fat retention would vary as function of the presence of saliva. Therefore, we argue that saliva does not play a substantial role in emulsion droplet adhesion and spreading.

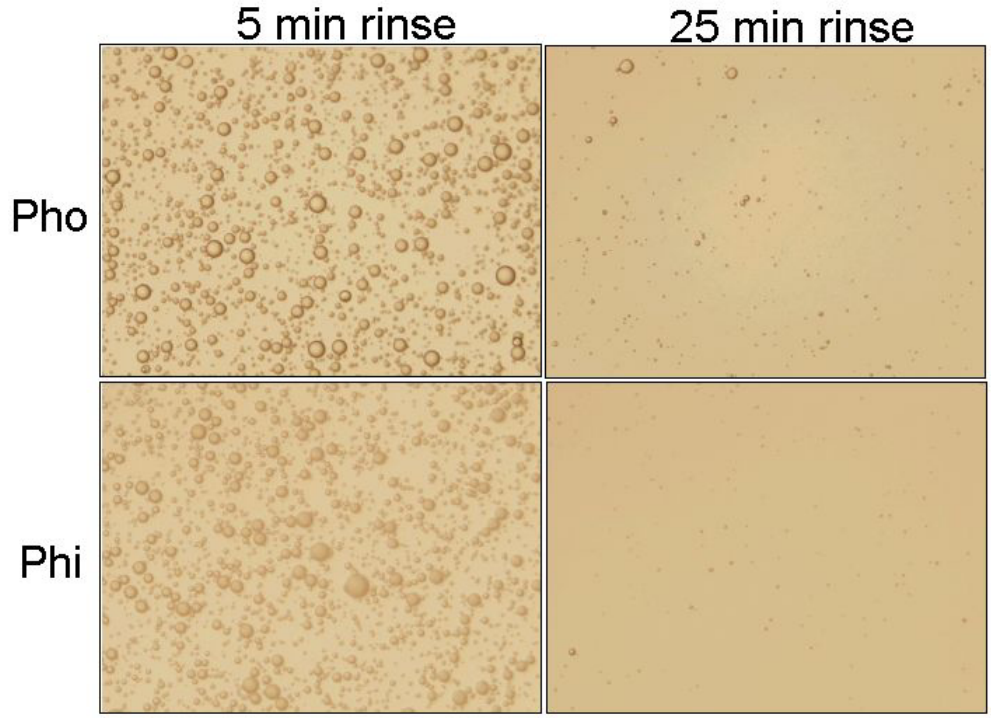

Figure 6.6: The influence of presence of large quantities of unstimulated saliva on adherence and spreading of an unstable (0.3% WPI) emulsion on hydrophobic (Pho) en hydrophilic (Phi) surfaces in time.

Table 6.2 Water contact angles of uncoated and saliva coated glass surfaces differing in initial hydrophobicity

	Water contact angle	
Surface	No saliva	Saliva
Pho	98.4 ± 0.43	10 ± 10
Phi	<3	39.3 ± 2.2

6.4 Conclusion

Fat perception has been shown in previous work to be dependent on the ability of the emulsion to form a lubricating layer and thus the ability to lower the (in-mouth)

friction. In the present work, we clearly show that adhesion and also spreading of emulsion droplets at the solid surface occurs. While spreading of (protein-) stabilised droplets at solid surfaces is a phenomenon that is sparsely described in literature, it is presumably a very important aspect in lubricating surfaces with emulsions.

We confirmed earlier preliminary indications that protein-poor emulsion droplets do adhere and spread more than protein-rich emulsions. The reason for this enhanced adherence and spreading of protein-poor droplets is shown to be related to a reduced electrostatic and steric and increased hydrophobic interaction. Especially the latter interaction was found to be crucial for o/w emulsion droplets adhesion and spreading.

Saliva has been shown to play a minor role in emulsion droplet adhesion and spreading at a solid surface. However, saliva does interact with the droplets and is able to remove droplets from the surface, thus cleansing the tongue. Using the obtained insight in droplet/solid surface interactions, which are of importance for spreading, these results give some first hints on how to design emulsions that are stable in bulk, but nevertheless spread on the tongue surface, thus lowering in-mouth friction.

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Summary and Outlook

For years, reducing the amount of fat in food products without losing the appreciated creamy/fatty sensation during consumption has been a challenge for both food industry and food science. We have seen the development of various fat-associated aromas, texturizing agents and fat-mimicking ingredients. Despite these efforts, however, a complete match with the full fat original products could not be reached. Fundamental understanding on how fat is perceived was largely lacking. All fat reduction measures seemed to miss some crucial, but unknown aspect related to the mouth-feel of the food product. This aspect is apparently not included in the rheological behaviour of the food product as such. The starting hypothesis of this thesis was that this unknown aspect of fat perception was related to a specific interaction of emulsified fat with oral surfaces. Relating sensory (fat) perception to physico-chemical interactions of emulsions with (oral) surfaces is a new research area. Therefore, a substantial part of the work described in this thesis concerns characterisation of the oral surfaces and emulsion systems, development of new techniques and methods, and identification of hypothesised dependencies between fat-perception and in-mouth friction.

In **Chapter 2,** we first of all introduce the mouth-mimicking tribometer, the so-called Optical Tribological Configuration (OTC). This tribometer was developed to test the hypothesis that there is a relation between interaction of emulsions with oral surfaces, in-mouth friction and fat perception. The OTC combines mouth-mimicking friction measurements with simultaneous observations of emulsion behaviour using a Confocal Scanning Laser Microscope (CSLM). Pig's tongue is used to mimic the surface of the human tongue, as the two have similar characteristics. Drawbacks of using 'real' tongue tissue are (i) the limited availability, (ii) the individual differences between the tongues, and (iii) the relative fast degradation of the tissue. To perform reliable friction measurements, preservation and preparation methods had to be developed.

The pig's tongue was characterized in terms of surface deformability, hydrophobicity and roughness. These surface characteristics are expected to influence the friction.

Furthermore, the characteristics of pig's tongue were compared to an artificial surface, namely polydimethylsiloxane (PDMS), which has been used as a mouth-mimicking surface by several other research groups. It was shown that the tongue has an average asperity height of several hundreds of micrometers, that it is hydrophobic (after removal of saliva from the surface), and that it has an elastic modulus two orders of magnitude lower than commonly applied PDMS. Furthermore, friction measurements have been performed while simultaneously observing emulsion droplet behaviour under shear using a CSLM. These friction measurements in combination with the CSLM observations reveal that the differences in surface characteristics between pig's tongue and smooth PDMS result in different tribological as well as emulsion coalescence behaviour.

Whether the observed differences in occurrence of coalescence described in Chapter 2 also correlate with sensorial differences in fat perception and in-mouth perceived roughness, is studied in **Chapter 3**. A series of emulsions with a variation in expected sensitivity towards (in-mouth) coalescence was prepared. The expected sensitivity was largely confirmed by both *in vivo* and *ex vivo* measurements. Sensorial evaluation using a Quantitative Descriptive Analysis (QDA) method revealed that emulsions with a higher sensitivity towards coalescence were perceived as lower in friction and higher in fatty/creaminess. Using the OTC and pig's tongue, we verified whether variations in sensitivity towards coalescence also correlate with differences in measured friction. The mouth-mimicking friction measurements showed that a lower *perceived* friction is indeed related to a lower physically *measured* friction. Furthermore, indications are found that both shear-induced coalescence and surface-induced coalescence (spreading of emulsion droplets on solid surfaces) play a role in lowering of in-mouth friction and thus increasing fat perception.

In **Chapter 4** we investigated in more detail how emulsions can lubricate surfaces

by varying both emulsion and surface characteristics. To this end, friction measurements are performed in the OTC, using PDMS surfaces modified in terms of surface roughness, deformability and hydrophobicity. We find that the modified PDMS surfaces in our experiments are boundary/mixed lubricated. This implies that the asperities on the surfaces are (largely) in contact. Presumably, the same applies to tongue/palate lubrication in the mouth. Boundary lubrication is determined by the chemical characteristics of the molecular (thin) layer of lubricant covering the asperities. This differs from hydrodynamic lubrication, since here the bulk viscosity of the lubricant determines the friction, and the asperities are not in contact. Emulsions, which are more sensitive towards coalescence, were found to be more effective in lowering friction than less sensitive emulsions. Most likely, spreading of emulsion droplets on solid surfaces (surface-induced coalescence) plays a role in lowering of the friction. Salivary protein and free protein in the continuous phase greatly enhanced the friction. Surprisingly, increasing the fat content (in the range 1 wt%- 40 wt%) of the emulsions had no effect on the *measured* friction. On the other hand, an effect of fat content on sensorial *perceived* roughness would be expected, since high amounts of oil would promote prolonged formation of lubricating layers, thus counteracting the removal of such layers by saliva. The mechanism underlying this effect was further investigated in Chapter 5.

Chapter 5 resumes the hypothesis developed in Chapter 4 that the ability of emulsions to spread on the tongue or tongue mimicking surfaces is essential for reducing friction. We investigated whether emulsions more sensitive towards coalescence can form lubricating layers, which contain more fat than less sensitive emulsions. *In vivo* and *ex vivo* measurements were performed on (oral) retention of fat. Furthermore, the role of saliva on fat retention was investigated. Emulsion droplets are proposed to attach to the tongue surface through adhesion and subsequent spreading of these droplets. Saliva is shown to play a minor role in the formation of a fat lubricating layer, but is of importance in removing oil from the hydrophobic tongue surface. Both *in vivo* and *ex vivo* measurements showed that protein-poor emulsion droplets were retained more by the tongue surface than protein-rich droplets. This is consistent with the expected larger surface forces between protein-poor droplets and the tongue surface as compared to

protein-rich droplets.

In **Chapter 6** we attempt to analyse the surface forces further, by visualising adhesion and spreading of emulsion droplets. The aim was to confirm dependencies of emulsion droplet adhesion on electrostatic, steric and hydrophobic interactions of emulsion droplets with the surfaces, as proposed in Chapter 5. Combining a flow cell with light microscopy and a CCD camera allowed us to distinguish between adhered and spread emulsion droplets. Using this method we confirmed earlier indications (Chapter 4 & 5) that protein-poor emulsion droplets do adhere and spread more than protein-rich droplets. It is demonstrated by varying the ionic strength and the hydrophobicity of the surface that this enhanced adherence of droplets is due to a reduced electrostatic (and steric) and increased hydrophobic interaction of the droplets with the surface. Other studies have shown that saliva interacts with the droplets. However, this interaction plays a minor role in emulsion droplet adhesion and spreading at the solid surface. These results provided some first ideas on how to engineer emulsions that are stable in the bulk, but nevertheless could spread more at the hydrophobic (tongue) surfaces. Slightly increasing hydrophobic interaction between droplets and oral surface, in combination with a reduced electrostatic and steric interaction should result in lowering of in-mouth friction, without affecting bulk stability too much.

Discussion and outlook

During the years this thesis work was performed, the interest in how to successfully develop low-fat food emulsions with an appreciated taste has grown, as well as the awareness that the answer possibly lies in combining product development with mouth-mimicking measurements. In the thesis work some first essential steps are made in characterising mouth-relevant surface characteristics and conditions. This information has been applied in developing mouth-mimicking equipment dedicated to understanding fat perception. The equipment is used in combination with various methods and techniques originally used in research areas differing greatly from the food science area. This combination of methods and approaches of various research areas led to the identification of some physico-chemical requirements a food emulsion should meet to be perceived as

'creamy' or 'fatty'.

In the thesis work we give evidence that the process, which is crucial for lowering in-mouth friction and thus enhancing fat perception is formation of an oil layer on the tongue surface. The oil layer will be formed when emulsion droplets adhere to the surface and subsequently spread, wetting the hydrophobic tongue. Emulsion droplets with a decreased stability against coalescence are more prone to adhere and spread on the solid surface.

On the risk of stating the obvious, to my opinion it is inevitable that the follow-up of this research also needs an interdisciplinary approach. Ideally, the findings of this research (performed with model food emulsions) would be directly applicable for commercial food products. However, there are obvious differences between the model systems and these products in terms of heat treatment, amount and type of different ingredients, and requirements for stability, which makes direct translation difficult. Furthermore, more detailed information is needed on the colloidal forces involved in droplet adhesion and spreading, as well as on the importance of the characteristics of the protein-stabilised interfacial layer, before we can apply the concept of 'controlled release' of fat to commercial products. Therefore, as continuation of this thesis work, there is a need for product developers to work in close collaboration with colloid scientists to develop emulsions, which are stable in bulk, during processing and shelf-life, and yet can spread easily on the tongue, thereby enhancing fat perception. To determine the effect on fat perception, sensory and tribological experiments remain of importance, and therefore an interdisciplinary approach is a necessity.

Starting point for follow-up research should be to determine in more detail which colloidal forces are involved in droplet adhesion and spreading. The flow cell, in combination with a surface force type apparatus, suitable for studying the interaction potential between hydrophobic surfaces and protein-stabilised emulsion droplets, could give useful information. Product developers could use such information in the choice for a specific emulsifier, pH, protein concentration to design emulsion droplets prone to spread on the tongue.

For commercial applications it is of importance to check how thickening (or even gelling) the continuous phase influences droplet spreading and in-mouth friction, since

thickening agents are often used in commercial food emulsions. Mouth-mimicking tribological measurements can play an essential role in evaluation of such emulsions. Optimising the characteristics of the mouth-mimicking surfaces, as used in the OTC, would increase the predictive value of these friction measurements. Measuring at body temperature would add to the mouth-mimicking quality of these tribological measurements. Sensorial tests are needed to verify the predicted capacity of emulsions to enhance fat perception.

In connection to thickening emulsions it would also be relevant to study the kinetics of droplet spreading as well as the influence of thickening of the continuous phase on these kinetics, since fat perception typically takes place at small time scales (several seconds).

Another subject, which is touched upon in this thesis and deserves attention in follow-up research, is the influence of aroma release on fat perception. Aroma has undoubtedly a large influence on fat perception. It would add to our understanding of fat perception if we could determine whether or not spreading of emulsion droplets on oral surfaces also influences aroma release. It is even more essential to define the balance between mouth-feel and aroma perception in total fat perception (for the different products). Hence, this determines in which type of food products controlled release of fat potentially can enhance fat perception by lowering of in-mouth friction.

In summary, the follow-up of this research should focus on developing emulsions, which should meet the requirement: “stable for long, but spreads at the tongue”. An example of this concept, which has been shown to be successful, is using hydrolysed starch (OSA, Chapter 3) to stabilise the emulsion droplets. These droplets were stable outside the mouth, but coalesced in the mouth due to breakdown of OSA by salivary amylase, resulting in an enhanced fat perception. This is one example of specific ‘mouth-induced’ destabilisation, which could give possibilities to reduce fat levels in food emulsions.

Samenvatting

Het is voor de wetenschap en de levensmiddelenindustrie al jarenlang een uitdaging om de hoeveelheid vet in levensmiddelen te verlagen zonder dat de lekkere romige smaak van het product te erg wordt aangetast. Er zijn dan ook al veel ingrediënten ontwikkeld die bijvoorbeeld de textuur of het aroma dat vet aan levensmiddelen geeft nabootsen. Ondanks de toepassing van deze ingrediënten in laag vette producten, konden nog geen goede overeenkomsten worden verkregen met vol vette producten. De fundamentele kennis over *hoe* mensen vet waarnemen was op dat moment nog grotendeels afwezig. Het had er alle schijn van dat de textuur verhogende ingrediënten die werden toegevoegd om te compenseren voor het verlagen van vet, een onbekend, maar cruciaal mondgevoel gerelateerd aspect van vetbeleving, niet konden nabootsen. Blijkbaar gaat het niet alleen om het reologische gedrag (viscositeit) van het product, maar is er nog een ander aspect dat belangrijk is in vet-perceptie. De starthypothese van dit proefschrift was dat dit onbekende aspect van sensorische vetperceptie een relatie heeft met een specifieke interactie van geëmulgeerd vet, oftewel vet omgeven door een eiwit laagje, met orale oppervlakken zoals de tong. Met het leggen van relaties tussen sensorische (vet) perceptie en fysisch chemische interactie van emulsies met (orale) oppervlakken betraden we een nieuw onderzoeksterrein. Vandaar dat een substantieel gedeelte van het werk beschreven in dit proefschrift betrekking heeft op het in kaart brengen van karakteristieken van de orale oppervlakken en emulsies, de ontwikkeling van nieuwe meetsystemen en het identificeren van verwachte afhankelijkheden tussen vetperceptie en frictie (wrijving) in de mond.

In **Hoofdstuk 2** introduceren we allereerst de mond-imiterende tribometer, de zogenaamde “Optical Tribological Configuration” (OTC). Deze tribometer is ontwikkeld om de hypothese te testen dat er een relatie is tussen de interactie van emulsies met orale oppervlakken, frictie en vetperceptie in de mond. Met de OTC kunnen mond-imiterende frictiemetingen worden uitgevoerd, terwijl tegelijkertijd de gedragingen van de emulsiedruppels worden bestudeerd met behulp van een “Confocal Scanning Laser

Microscope" (CSLM). Om humane tongen te imiteren zijn varkenstongen gebruikt, aangezien beide vergelijkbare karakteristieken hebben. Nadeel van het gebruik van 'echt' tongweefsel is (i) de beperkte beschikbaarheid, (ii) de individuele verschillen tussen de tongen, (iii) de relatief snelle degradatie van het weefsel. Om betrouwbare metingen te kunnen uitvoeren, moesten daarom preservatie- en preparatiemethoden ontwikkeld worden. De varkenstong is gekarakteriseerd op oppervlakte vervormbaarheid, hydrofobiciteit en ruwheid. De verwachting is dat deze oppervlaktekarakteristieken een invloed hebben op frictie.

Daarnaast zijn de karakteristieken van de varkenstong vergeleken met die van een kunstmatig oppervlak, namelijk polydimethylsiloxaan (PDMS). Dit type materiaal is al eerder gebruikt als mond-imiterend oppervlak door andere onderzoeksgroepen. Oppervlakte-karakterisering laat zien dat de tong oneffenheden heeft die een gemiddelde lengte hebben van enkele honderden micrometers, dat de tong hydrofoob is (na verwijdering van speeksel) en dat de tong honderd keer zo vervormbaar is als een PDMS oppervlak (Youngs modulus 100 keer zo laag). Bovendien zijn er frictiemetingen uitgevoerd terwijl tegelijkertijd het gedrag van de emulsiedruppels onder afschuiving met behulp van de CSLM is bestudeerd. Deze combinatie van frictiemetingen en CSLM-observaties, toont aan dat de verschillen in oppervlaktekarakteristieken tussen varkenstong en glad PDMS resulteren in verschillend tribologisch (andere frictie) en emulsie coalescentie gedrag. Het samenvloeien van twee druppels, in dit geval emulsiedruppels, wordt ook wel coalescentie genoemd.

Of de in Hoofdstuk 2 waargenomen verschillen in het optreden van coalescentie ook correleren met sensorische verschillen in vet-perceptie en sensorisch waargenomen ruwheid in de mond, is verder onderzocht in **Hoofdstuk 3.** In de beschreven experimenten zijn emulsies gebruikt waarvan de verwachting is dat er verschil is in gevoeligheid voor coalescentie in de mond. *In vivo* (dwz. in de mond) en *ex vivo* (buiten de mond) metingen bevestigden dat deze verwachting grotendeels juist was. De sensorische evaluatie van de set emulsies, met behulp van de "Quantitative Descriptive Analysis" (QDA) methode, laat zien dat hetzelfde verband tussen type emulsie en hoogte van de frictie door mensen sensorisch wordt waargenomen als *ex vivo* met de OTC wordt gemeten. Daarnaast zijn er

aanwijzingen gevonden dat zowel afschuiving geïnduceerde coalescentie als oppervlakte geïnduceerde coalescentie (dus spreiding van emulsiedruppels op vaste oppervlakken) een rol spelen in het verlagen van de in de mond waargenomen frictie en dus in het verhogen van de vetperceptie.

In **Hoofdstuk 4** hebben we in meer detail onderzocht *hoe* emulsies oppervlakken kunnen smeren (in het Engels "lubricate") door zowel emulsie- als oppervlaktekarakteristieken te variëren in frictie-experimenten. PDMS-oppervlakken zijn dusdanig gemodificeerd dat oppervlakken zijn ontstaan verschillend in ruwheid, vervormbaarheid en hydrofobiciteit. Door frictiemetingen uit te voeren met behulp van deze PDMS-oppervlakken hebben we aangetoond dat de oppervlakken grens- en/of gemengd gesmeerd worden, zelfs als de viscositeit van het smeermiddel (lubricant) erg hoog is. Dit houdt in dat de oneffenheden op de oppervlakken tijdens het over elkaar wrijven grotendeels in contact met elkaar zijn. Naar alle waarschijnlijkheid treedt grens- en/of gemengde smering ook op wanneer de tong over het verhemelte wrijft. Grenssmering wordt bepaald door de chemische karakteristieken van de moleculaire (en dus zeer dunne) smeermiddellaag die de oneffenheden bedekt. Dit is fundamenteel anders dan wanneer volle film smering (hydrodynamisch) smering optreedt en de oneffenheden *niet* met elkaar in contact zijn. Emulsies, die meer gevoelig zijn voor coalescentie verlaagden de frictie meer dan emulsies die minder gevoelig waren. Hoogstwaarschijnlijk speelt het spreiden van emulsiedruppels op vaste oppervlakken (oppervlakte-geïnduceerde coalescentie) een rol bij de verlaging van de frictie. Speeksel eiwit en 'vrij' eiwit (eiwit in de continue fase) verhogen de frictie aanzienlijk. Verrassend genoeg heeft het verhogen van het vetgehalte van de emulsies (in het bereik 1 gew.% - 40 gew.%) geen effect op de gemeten frictie. Aan de andere kant is wel de verwachting dat vetgehalte een effect heeft op de sensorisch waargenomen frictie in de mond, aangezien een hoog vetgehalte de langdurige vorming van smerende lagen op het oppervlak bevordert en dus de permanente verwijdering van de smeringslagen van het tongoppervlak door speeksel uitstelt.

Hoofdstuk 5 grijpt terug op de hypothese geformuleerd in Hoofdstuk 4 dat de potentie van emulsies om te spreiden op de tong of tong-nabootsende oppervlakken

essentieel is voor het verlagen van de frictie. We hebben onderzocht of emulsies die meer gevoelig zijn voor coalescentie daadwerkelijk smeringslagen kunnen vormen, die meer vet bevatten dan emulsies die minder gevoelig zijn voor coalescentie. *In vivo* en *ex vivo* metingen zijn uitgevoerd om te bepalen hoeveel vet achterblijft op de oppervlakken (retentie van vet). Bovendien is de rol van speeksel in de retentie van vet op de tong onderzocht. We verwachten dat emulsiedruppels aan het tongoppervlak hechten via zowel adhesie als daaropvolgend spreiding van de druppels. We laten zien dat speeksel een minder grote rol speelt bij de vorming van een smeringslaag, maar dat speeksel wel belangrijk is bij het verwijderen van vet (of olie) van de hydrofobe tong. Zowel *in vivo* als *ex vivo* metingen laten zien dat "eiwit-arme" emulsiedruppels (oftwel emulsiedruppels gestabiliseerd door kleine hoeveelheden eiwit) meer achterblijven op het tongoppervlak dan "eiwit-rijke" emulsiedruppels. Dit is in overeenstemming met de verwachting dat oppervlaktekrachten tussen eiwit-arme druppels en de tong groter zullen zijn dan de krachten tussen de tong en eiwit-rijke druppels.

In **Hoofdstuk 6** pogen we de aard van de oppervlaktekrachten verder te analyseren door experimenten uit te voeren waarbij adhesie en spreiding van emulsiedruppels via een directe methode bestudeerd wordt. Het doel is aan te tonen dat het optreden van adhesie en spreiding van druppels afhangt van de electrostatische, sterische en hydrofobe interactie tussen emulsiedruppels en de vaste oppervlakken, zoals al eerder gesuggereerd in Hoofdstuk 5. Het combineren van een stromingscel (flow cell) met een CCD-camera schiep de mogelijkheid om intacte druppels die aan het oppervlak gehecht waren door adhesie te onderscheiden van druppels die reeds gespreid waren. Met behulp van deze methode konden we eerdere indicaties (zie Hoofdstuk 4 & 5), dat eiwit-arme emulsiedruppels meer hechten èn spreiden dan eiwit-rijke druppels, bevestigen. Door variaties aan te brengen in de ionsterkte en de hydrofobiciteit van het oppervlak is aangetoond dat de verhoogde adhesie van eiwit-arme druppels te danken is aan een verlaagde electrostatische (en sterische) en verhoogde hydrofobe interactie van de druppels met het oppervlak. Andere studies hebben aangetoond dat speeksel interacties aan kan gaan met emulsiedruppels. Deze interactie speelt echter slechts een kleine rol in adhesie en spreiding van emulsiedruppels op een vast oppervlak. Deze resultaten geven

wat eerste ideeën over hoe emulsies ontwikkeld kunnen worden, die stabiel zijn in de bulk, maar toch kunnen spreiden op hydrofobe (tong)oppervlakken. Een kleine toename in hydrofobe interactie tussen druppels en orale oppervlakken, in combinatie met een afname in electrostatische en sterische interactie, zou kunnen resulteren in een verlaging van de frictie in de mond, zonder teveel de bulkstabiliteit van de emulsie aan te tasten.

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Diane

List of Publications

- Dresselhuis, D. M., de Hoog, E. H. A., Cohen Stuart, M. A., van Aken, G. A. (2007). Tribology as a Tool to Study Emulsion Behaviour in the Mouth. In Dickinson, E. & Leser, M. E., *Food Colloids: Self-Assembly and Material Science*. (pp. 451-461). Cambridge: RSC Publishing.

- Dresselhuis, D. M., de Hoog, E. H. A., Cohen Stuart, M. A., van Aken, G. A. (2008). Application of oral tissue in tribological measurements in an emulsion perception context. *Food Hydrocolloids*, 22(2), 323-335.

- Dresselhuis, D. M., de Hoog, E. H. A., Cohen Stuart, M. A., van Aken, G. A. The occurrence of in-mouth coalescence of emulsion droplets in relation to perception of fat. *Food Hydrocolloids, DOI:10.1016/j.foodhyd.2007.06.013*, in press.

- Dresselhuis, D. M., Klok, H. J., Cohen Stuart, M. A., de Vries, R. J., van Aken, G. A.,de Hoog, E. H. A. (2007). Tribology of o/w emulsions under mouth-like conditions: determinants of friction. *Food Biophysics*, 2(4), 158-171

- Dresselhuis, D. M., Cohen Stuart, M. A., van Aken, G. A., Schipper, R. G., de Hoog, E. H. A. Fat retention at the tongue and the role of saliva: adhesion and spreading of stable versus unstable emulsions. submitted.

- Dresselhuis, D. M., van Aken, G. A., de Hoog, E. H. A., Cohen Stuart, M. A. Direct observation of adhesion and spreading of emulsion droplets at solid surfaces: influence of ionic strength and amount of emulsifier. submitted.

- de Hoog, E. H. A., Prinz, J. F., Huntjens, L., Dresselhuis, D. M.,van Aken, G. A. (2006). Lubrication of oral surfaces by food emulsions: the importance of surface characteristics. *Journal of Food Science*, 71(7), E337-E341.

Curriculum vitae

Diane Margriet Dresselhuis werd geboren op zondag 19 februari 1978 te Delfzijl. Haar VWO-diploma behaalde ze in 1996 aan het Ommelander College te Appingedam. In datzelfde jaar begon Diane aan de studie levensmiddelentechnologie aan de Wageningen Universiteit. Haar eerste afstudeervakonderzoek werd uitgevoerd bij de leerstoelgroep Levenmiddelenchemie met als standplaats Numico Research te Wageningen en ging over de scheiding en toepassing van ACE-inhiberende peptiden. Stage liep Diane bij Fonterra Research (toen NZDRI) in Palmerston North, Nieuw Zeeland. Een tweede afstudeervak werd uitgevoerd bij de vakgroep Toxicologie naar het effect van groente en fruit op expressie van genen die betrokken zijn bij het ontstaan van darmtumoren. Tenslotte liep Diane stage bij Heinz BV in Nijmegen (toen nog Honig). In januari 2002 studeerde ze af waarna ze nog korte tijd bij Heinz in Nijmegen werkte. Vervolgens begon ze in mei 2002 als junior onderzoeker bij Numico Research als ontwikkelaar klinische voeding. In november 2003 begon zij haar promotieonderzoek bij het laboratorium voor Fysische chemie en Kolloidkunde aan de Wageningen Universiteit. Voornamelijk hier, maar ook gedeeltelijk bij NIZO Food Research te Ede, voerde ze, in opdracht van het Wageningen Centre for Food Sciences, het in dit proefschrift beschreven werk uit. Sinds 1 november 2007 is ze werkzaam bij Friesland Foods Corporate Research in Deventer als onderzoeker in de groep “Food Structuring”.

Overview of completed training activities

Discipline specific activities

International advanced course on Industrial proteins	VLAG	2003
18th conf. of the European Colloid and Interface Society	Almeria, Spain	2004
Rheology workshop	Anton Paar	2004
Autumn meeting on macroscopic Physical Chemistry, (Physical Chemistry and Colloid Science)	WUR	2004
Liquids and Interfaces	NWO	2004
Intr. to Soft condensed Matter & Advanced Colloid Science	Utrecht University	2005
1st Int. Symp. on deliv. of Funct. in Complex Food Syst. (Nestlé and University of Fribourg)	Lausanne, Switzerland	2005
BASF PhD trip*	Ludwigshafen, Germany	2005
Food Colloids*	Montreux, Switzerland,	2006
Tribology Workshop*	WCFS	2006
5th NIZO Dairy Conference*	NIZO	2007
2nd Int. Symp. on deliv. of Funct. in Complex Food Syst*. (University of Massachusetts)	Amherst, USA	2007

General courses

VLAG PhD week	VLAG	2004
Scientific writing	WUR	2005
Debating course	WCFS	2006
Career perspectives (Wageningen Graduate Schools)	WUR	2007

* Oral presentation

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