

# Lubrication & Perception of foods

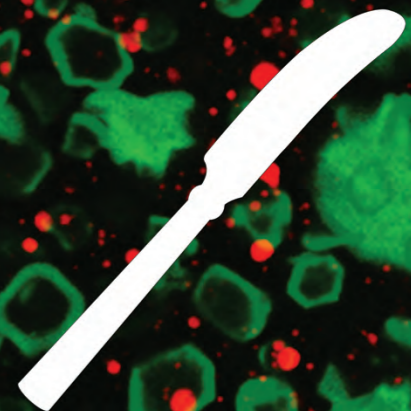
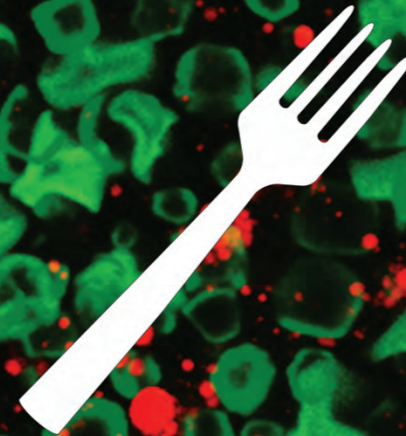


Lubrication & perception of foods

Kun Liu 2016



# Lubrication & Perception of foods



# **Lubrication and perception of foods**

**Tribological, rheological and sensory properties  
of particle-filled food systems**

**Kun Liu**

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# **Lubrication and perception of foods**

## **Tribological, rheological and sensory properties of particle-filled food systems**

**Kun Liu**

### **Thesis**

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*Per aspera ad astra*

Through difficulties to the stars

循此苦旅 以达天际

***To my parents***





# Abstract

## Background and aims

Food structure is determined by its composition and the interaction between the compositional or structural elements. Both food structure and the texture perception of foods undergo dynamic changes during different phases of oral processing. During oral processing, both rheological and tribological properties of foods are relevant for sensory perception. The general aim of this thesis was to understand the relationship between the structural properties, rheological and tribological properties during food breakdown, and the sensory perception of foods. More specifically, this thesis aimed to link the properties of food particles in liquid and semi-solid matrices to the tribological and rheological properties, and in this way, understand the sensory perception of these systems.

## Methods

Fat droplets and micro-particle fat replacers based on protein and starch were investigated. These particles varied in size, morphology, deformability and stability, as well as their interaction with the surrounding matrix. These particles were dispersed in liquid or semi-solid gel phases, forming the food model systems under consideration. The friction and microstructural evolution of food model systems under shear was determined using a mouth-mimicking tribometer connected to a confocal laser scanning microscopy. The viscosities of liquid systems were analyzed using a rheometer, and the large deformation properties of semi-solid gel systems were determined during uniaxial compression tests. The sensory perception of the food model systems were measured using quantitative descriptive analysis. The release and deposition of fat droplets on the tongue were determined using *in vivo* fluorescence.

## Results

Food structural elements could be manipulated to control the tribological properties of food model systems. Morphology, size, and deformability of food particles determine the lubrication behavior of the food systems. Spherical particles with micrometer size were able to reduce friction through a ball bearing mechanism, while irregularly shaped particles increased friction by increasing apparent surface asperity contacts. Deformable particles could flatten the surface by filling asperities, thus reduced friction. Coalescence of unstable droplets could plate-out on the surface and form film patches, thus reduced friction. Other structural elements, such as emulsifiers and sticky molecules, also influenced tribological

properties of the systems. Interactions between the food structural elements could influence the rheological properties of liquid and semi-solid food systems. These properties as well as tribological properties were inter-related and all of them affect sensory perception. The inter-relationships between physical and sensory properties of food systems were influenced by oral processing, such as oral processing duration and temperature. Furthermore, several fat reduction and replacement strategies were suggested, including increasing the availability of fat that is in contact with oral surfaces, improving the lubrication by ball bearing of particles, and reducing perception of negative attributes such as roughness.

## **Conclusions**

This thesis showed the importance of food particle properties in both the tribological properties and sensory perception of foods, and emphasized the different lubrication mechanisms of different structure elements and their relation to perception. The differences in behavior of food particles between liquid and semi-solid gel systems were highlighted. These findings would enable a better understanding of relationship between food structure and their physical and sensory properties, and this would allow designing or modifying food products with targeted texture and sensory perception.

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# Chapter 1

## General introduction

## 1.1 Introduction & overall aim

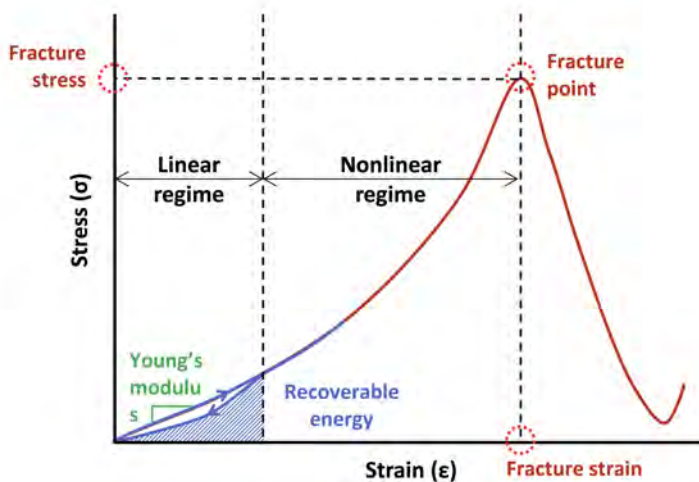
Understanding the relationship between food structure and texture perception can help improve the design of food products tailored to specific consumer preferences. The perception of food texture is strongly influenced by its composition, structure, and the dynamic oral processing (Dickinson, 2012; Hutchings & Lillford, 1988; Stokes *et al.*, 2013). During oral processing, foods undergo continuous structural changes, including breakdown into smaller fragments, release of nutrients and formation of a cohesive bolus by saliva incorporation, and formation of oral coating after swallowing (Brandt *et al.*, 1963; Foegeding *et al.*, 2011; Stokes *et al.*, 2013). Both rheological and tribological properties of the food at different oral processing phases have been demonstrated to influence texture perception (Bellamy *et al.*, 2009; van Aken *et al.*, 2011), and the oral processing entails a “transition from a rheology-dominant process to a tribology-dominant process” (Stokes *et al.*, 2013). In order to have a comprehensive understanding of the influence of the food ingredients and structure on sensory perception, it is of importance to consider both the aspects of rheology and tribology.

## 1.2 Rheology

Rheology is the study of flow and deformation of matter. It applies to liquid, semi-solid, and solid materials under applied forces. The rheological behavior of liquid foods is often characterized by shear viscosity, which is defined as the ratio between shear stress and shear rate. Liquids that show a linear relation between shear stress and shear rate are called Newtonian. Most foods, however, show a non-Newtonian behavior, and most commonly, shear thinning, where the shear viscosity decreases with increasing shear rate. The viscosity of liquid foods influences their texture perception, such as thickness (Malone *et al.*, 2003; Richardson *et al.*, 1989). In addition, viscosity also influences the lubrication properties of liquid systems (see section 1.3).

The rheological properties of semi-solid systems are characterized in a small and large deformation regime. Large deformation measurements were found to be more relevant to texture and sensory properties (Foegeding & Drake, 2007; Luyten *et al.*, 1992). The most commonly used mode of deformation is uniaxial compression since it resembles part of the human mastication process and measures material properties (Roudaut *et al.*, 2002; Sala, 2007). During the deformation of food systems, stress ( $\sigma$ ) and strain ( $\epsilon$ ) are used to describe the deformation behavior. Stress is defined as force per unit area causing the deformation, and strain is defined as the ratio of length of deformation versus initial length as caused by the

applied force. The relationship between stress and strain has often a shape as shown by the stress-strain curve in Figure I - 1. When the relationship between stress and strain is linear, the slope of the stress-strain curve is defined as the elastic modulus, or Young's modulus. Recoverable energy can be determined by compressing and de-compressing the solid gel within the linear regime (van den Berg *et al.*, 2008a). At a high degree of deformation, the gel will fracture. At this point, the stress and strain are defined as fracture stress and fracture strain. These mechanical properties under large deformation have been linked to sensory perception of food texture (Luyten *et al.*, 1992; Montejano *et al.*, 1985). Young's modulus can correlate to firmness and stiffness (Foegeding *et al.*, 2011; Kramer & Szczesniak, 1973), and recoverable energy can link to crumbliness (van den Berg *et al.*, 2008; van den Berg *et al.*, 2008b). Hardness is often found to correlate to fracture stress, and brittleness is often found to correlate to fracture strain (Devezeaux de Lavergne *et al.*, 2015; Gwartney *et al.*, 2004; Xiong *et al.*, 2002).



**Figure I - 1 Stress-strain relationship during large deformation of a semi-solid gel**

For semi-solid gels that contain particle fillers, the large deformation properties of the gels are influenced by the properties of the gel matrix, filler particles, as well as the interactions between the matrix and the particle fillers (Chen & Dickinson, 1998; Sala *et al.*, 2007a; van Vliet, 1988). The filler particles can be classified as either “active” or “inactive”, depending on their effects on the gel modulus (Chen *et al.*, 1998). Active fillers have strong interaction with the matrix, thus they are also considered “bound” to the gel matrix. Active fillers participate in the gel matrix network, thus the gel modulus is increased by fillers with higher

moduli than that of the matrix, and vice versa. Inactive fillers have little chemical nor physical affinity to the matrix material (“unbound” to the matrix) and hence always decrease the gel modulus (Ring & Stainsby, 1982; van der Poel, 1958). The interaction between filler and matrix is determined by the surface properties of the fillers. In the case of emulsion-filled gels, the interaction between the droplets and matrix is determined by the emulsifier that stabilizes the droplets (Sala *et al.*, 2007a).

### 1.3 Tribology

Tribology, also called “thin-film rheology”, is the study of friction, wear and lubrication of interacting surfaces in relative motion. Tribology is commonly applied in mechanical engineering and materials science. During recent years, tribology has been employed in food research as well. Under conditions relevant for oral processing of foods, the tongue and upper-palate represent two interacting surfaces in relative motion. These two surfaces are lubricated by a layer of saliva and food. The application of tribology in food research has revealed important correlations between lubrication properties and perception of foods (Chojnicka-Paszun *et al.*, 2012; Dresselhuys *et al.*, 2007; Kokini & Cussler, 1983).

Friction is the force resisting the motion of interacting surfaces during sliding and rolling. Friction between two surfaces without relative motion is called static friction. Static friction force must be overcome by an applied force in the direction of motion to initiate or sustain motion. Dynamic friction is the friction generated between two surfaces that are in relative motion. This thesis concerns friction during dynamic oral processing, thus only dynamic friction is considered. Friction is often expressed as a friction coefficient, which is defined as

$$\mu = F/W$$

where  $\mu$  is the friction coefficient,  $F$  is friction force, and  $W$  is the normal load perpendicular to the direction of friction. This equation, also called Amontons’ equation, is considered as the most basic rule of sliding friction (Amontons, 1699; Bhushan, 2013).

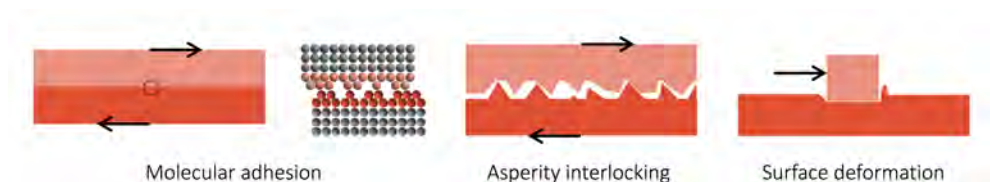


Figure 1 - 2 Origins of friction. (Inspired by Bhushan (2013) and Krim (2002)).

Friction principally originates from molecular adhesion, asperity interlocking and surface



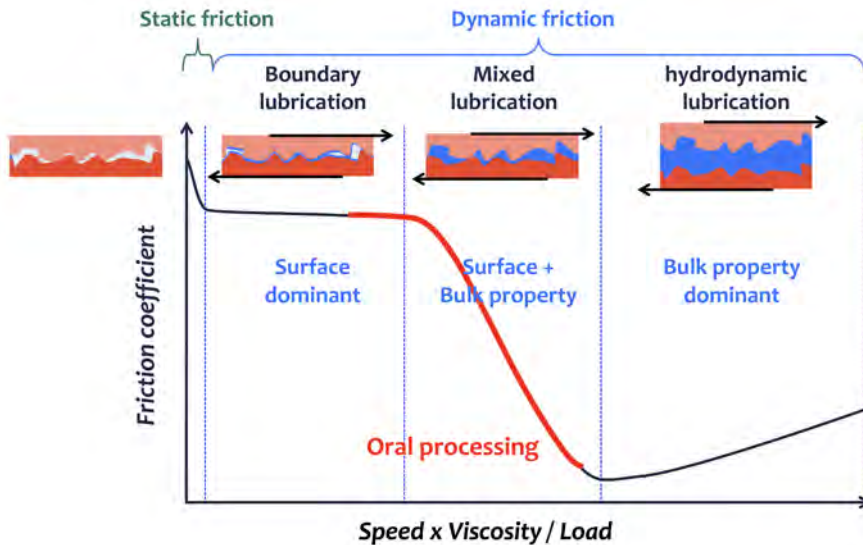
deformation (the plowing effect) (Figure I - 2). Thus, surface properties, such as adhesion, hydrophobicity, roughness, and deformability are very important factors that influence friction. When there is a fluid lubricant present in between the two surfaces, friction is also influenced by the characteristics of this lubricant and the interaction of the lubricant with the surfaces. In addition, depending on the speed of the surface motion, entrainment of the lubricant between the surfaces is influenced. The entrained lubricant can provide different support for the two interacting surfaces at different speeds. The friction behavior of lubricants is often represented in form of a so-called Stribeck curve, where the friction coefficient is plotted as a function of film thickness, or as a function of a so-called friction parameter (defined as speed x viscosity / load) (Figure I - 3).

The Stribeck curve contains three lubrication regimes. When the speed of the relative motion of surfaces is small (and the lubricant has low viscosity), there is not enough lubricant that enters and stays in between the two surfaces. In this case, the separation between the surfaces is small and the asperities of the surfaces are in direct contact. This regime is called the “boundary lubrication regime”. In the boundary regime, the friction force is highly related to surface roughness (asperity interlocking). With increasing surface roughness, more asperity contact will occur. This can be reflected as an extension of the boundary regime towards higher speed, since higher speed is needed to induce more lubricant entrainment and thus better surface separation. Thus, the span of the boundary regime provides information of the surface roughness. The height of the boundary regime (value of the friction coefficient) depends on the physicochemical properties of the surfaces and the chemical constitution of the thin lubricant films that covers the surfaces (Butt *et al.*, 2013). These lubricant films can have a thickness of a few molecules only.

With increasing speed of motion, more lubricant is entrained between the surfaces thus exerting higher supporting pressure that separate the surfaces better. In this case, only large asperities are in contact and friction is reduced. This behavior continues with further increasing speed, which is reflected in the Stribeck curve as a decreasing trend with increasing speed, known as the “mixed lubrication regime”. As can be read from the name, in the mixed regime both the properties of contact surfaces and the bulk properties of the lubricant are important for friction. Several studies have suggested that tribological behavior of foods during oral processing is essentially in the mixed lubrication regime (Chojnicka-Paszun *et al.*, 2012; Dresselhuis *et al.*, 2008b; Malone *et al.*, 2003).

Once the entrainment of lubricant is high enough due to increasing speed or bulk viscosity to separate the two surfaces fully, the Stribeck curve enters the “hydrodynamic lubrication

regime” (or elasto-hydrodynamic regime if soft surfaces are employed). In this regime, friction between the surfaces is mainly determined by the lubricant’s bulk properties, such as viscosity and structure.



**Figure 1 - 3 Stribeck curve and three lubrication regimes. Friction coefficient is plotted as a function of film thickness or a so-called friction parameter defined as speed x viscosity / load.**

Friction can be measured using a tribometer. Several types of tribometers are developed and used in the area of food research. The differences among these tribometers are often regarding the type and speed of the surface movements, contact area between the surfaces, and the surface materials. These parameters can be modified and adjusted to mimic the environment of the human mouth. For example, different from the smooth metal surfaces in conventional tribometers, which allows reaching very high speeds, soft materials, such as PDMS and pig tongues are used in food tribology applications (Bongaerts *et al.*, 2007; Dresselhuis *et al.*, 2008b). The surface roughness and hydrophobicity of the tribometer surfaces, as well as the lubrication function of saliva are also known to influence friction (Bongaerts *et al.*, 2007; Vardhanabhuti *et al.*, 2011).

Most studies on tribology in food research focus on liquid foods or liquid food model systems, such as milk (Chojnicka-Paszun *et al.*, 2012), cream (Baier *et al.*, 2009; Kokini *et al.*, 1983), protein dispersions (Chojnicka *et al.*, 2008), guar gum solutions (Malone *et al.*, 2003), starch, locust bean gum solutions (Zinoviadou *et al.*, 2008), and emulsions (Dresselhuis *et*

*al.*, 2007; Malone *et al.*, 2003). Among these liquid systems, emulsions have been studied extensively to understand the influence of fat droplets on lubrication. It has been found that in liquid emulsions, the sensitivity of oil droplets towards coalescence, was found to be correlated to lubrication and fat-related perceptions (Dresselhuis *et al.*, 2008c). In contrast to the extensive studies on liquid systems in relating microstructure with tribology and sensory, there are much fewer of these studies performed on semi-solid foods or food models. These studies include mayonnaise (de Wijk & Prinz, 2005), butter, cream cheese, ice cream (Kokini *et al.*, 1983), fluid gels (Garrec & Norton, 2013) and emulsion-filled gels (Chojnicka *et al.*, 2009). In general, these studies indicate that the individual components, interaction between the components, as well as the microstructure of the food systems can affect lubrication properties as well as sensory perception to a large extent. In this thesis, both liquid and semi-solid gel systems are investigated.

#### 1.4 Oral coatings

Oral coatings are food residues that stay on the oral surfaces after consumption of food and beverages. The composition and tribological properties of oral coatings can influence perception of taste, aroma and mouthfeel that are related to lubrication (de Wijk *et al.*, 2006b).

A limited number of methodologies to quantify the fat deposition of oral coatings has been suggested from literature. The efficiency of most methods, such as measuring the turbidity of spat-out (Prinz *et al.*, 2006), and determining coating composition using ATR FT-IR analysis (de Jongh & Janssen, 2007), is limited to an extraction step to collect the oral coating. The *in vivo* fluorescence method, developed by Pivk *et al.* (2008), provides a direct measure of fat deposited on the tongue and allows studying the formation and clearance dynamics of fat fraction in oral coatings over time without an extraction/collection step. Using this *in vivo* fluorescence method, the oil fraction deposited on the tongue after consuming oil or o/w emulsions was quantified (Camacho *et al.*, 2014; Pivk *et al.*, 2008). They reported that the oil fraction deposited on the tongue and the fat-related mouthfeel and afterfeel perceptions increased with increasing oil content in liquid o/w emulsions. Knowledge on the behavior and afterfeel perception of oral coatings formed by liquids has increased in recent years. However, studies describing oral coatings formed by semi-solid and solid foods are scarce. Previous studies have suggested that the release of fat from emulsion-filled gels and the coalescence of fat droplets under mouth-mimicking *in vitro* conditions lead to a decrease in friction, and to an increase in perception of fat-related sensory attributes (Dresselhuis *et al.*, 2007; Sala *et al.*, 2007b). Evidence for such an increase of the amount of fat deposited in the oral cavity

during oral breakdown of emulsion-filled gels due to increased fat release and coalescence is not available.

## 1.5 Sensory perception

As described earlier, the perception of food texture is a dynamic phenomenon during oral processing. For semi-solid gels, the attributes firmness and brittleness are usually perceived in the first phase. These attributes are primarily related to large deformation properties of foods (rheology dominated) (Brandt *et al.*, 1963). In the later phases (the masticatory phase) of oral processing, the food bolus is subjected to shear, extensional flow, and displacement, which are strongly influenced by both the rheological and tribological behavior of the bolus (Chen & Stokes, 2012; de Wijk *et al.*, 2006b). Mouthfeel attributes, such as chewiness, stickiness and creaminess, are often perceived in this phase (Brandt *et al.*, 1963). After swallowing the food bolus, food residues can remain adhered to oral surfaces to form an oral coating, which dominates the mouthfeel and afterfeel perception (Camacho *et al.*, 2014; de Wijk *et al.*, 2009a). Food residuals that adhere to oral surfaces are often sensed as creamy, smooth and velvety, or rough, dry, and gritty. The afterfeel perception is determined more by the tribological properties of food residuals and their interactions with saliva than the rheological properties of foods (Prakash *et al.*, 2013).

Creamy is often the most appreciated of all the relevant sensory attributes, and the most difficult to understand and maintain. It is a complex sensorial characteristic related to multiple food properties (Akhtar *et al.*, 2006). It has been suggested that creaminess can be predicted from thickness, smoothness and slipperiness. These properties, in turn, are mainly related to the viscosity and lubrication properties of foods in the mouth (Cussler *et al.*, 1979; Kokini, 1987; Kokini *et al.*, 1983). The melting of foods in the mouth can also influence the perception of creaminess, which might be related to the lubrication effect of the molten layer (Kokini, 1987). Next to these factors, the presence of particles also affects the creaminess perception (Kilcast & Clegg, 2002; Krzeminski *et al.*, 2013). Large and non-smooth particles added to foods are often associated with several unappreciated sensory attributes, such as rough, dry, gritty and powdery (Cheftel & Dumay, 1993; Krzeminski *et al.*, 2013; Petersson *et al.*, 2013). The perception of these attributes is caused by friction between the particles and the oral mucosa (de Wijk *et al.*, 2005; Hollins *et al.*, 2000), and they might suppress creaminess (de Wijk & Prinz, 2006a; Singer, 1996; Wood, 1974). In contrast, when the particles are small, soft, and are able to roll over each other, they contribute to creaminess perception. Furthermore, when the particles are too small, it is suggested that they are unable to provide a so-called impression of “substantialness”, and

might instead contribute to a “watery” perception (Singer, 1996). Detection of particles in the mouth is related to physical properties of the particles, such as size, concentration, shape, and hardness, as well as properties of the matrix in which the particles are dispersed. Additionally it depends on interactions between the particles and the matrix, oral tissues and the saliva (de Wijk *et al.*, 2005, 2006a; Engelen *et al.*, 2005a; Engelen *et al.*, 2005b; Heath & Prinz, 1999; Imai *et al.*, 1995; Kilcast *et al.*, 2002; Minifie, 2012; Petersson *et al.*, 2013; Sala & Scholten, 2015; Tyle, 1993). Therefore, the size threshold for the perception of particles embedded in a food matrix may depend on all factors mentioned above.

## **1.6 Food particles**

Practically most foods can be viewed as particles dispersed in a liquid phase or a semi-solid/solid phase. These particles can be from different sources, such as oil droplets and protein particles in milk, cheese and sausages, starch granules in puddings, cocoa and sugar particles in chocolate, fruit particles in applesauce and ketchup. Particles in foods exhibit a large range of distribution in size, shape and deformability. In this thesis, fat droplets and particles that have a potential to mimic the properties of fat droplets are investigated.

### **1.6.1 Fat droplets**

In foods, fats exist often in the form of droplets and mostly embedded in an aqueous phase, either liquid or semi-solid. Fats contribute to many aspects in determining the overall properties of foods, such as appearance, flavor, texture, mouthfeel, and palatability (Akhtar *et al.*, 2006; Chung *et al.*, 2013; McClements & Demetriades, 1998). Among these properties, the impact of fat on texture perception of foods, especially those related to perception of mouthfeel attributes, such as creaminess, is not fully understood. It was found that coalescence of fat droplets occurring during oral processing led to a decrease of friction and to an increase of perception of fat-related sensory attributes, such as creaminess (Chojnicka-Paszun *et al.*, 2012; Dresselhuis *et al.*, 2008a; Dresselhuis *et al.*, 2008c). The sensitivity of emulsion droplets towards coalescence under conditions mimicking oral processing behavior can be modified by changing the droplet size or solid fat content (SFC), adding unsaturated mono-glyceride, and by changing the emulsifier type and concentration (Benjamins *et al.*, 2009; Dresselhuis *et al.*, 2008c). In addition, as mentioned in section 1.2, when the fat droplets are embedded in a semi-solid gel, the droplets can be bound or unbound to the gel matrix depending on the emulsifier that adsorbed at the surface of the droplets. Different droplet-matrix interactions also have influence on the friction and the perception of fat-related attributes (Chojnicka *et al.*, 2009).

## 1.6.2 Particle fat replacers

There has been an increased consumer demand for healthier foods with lower amounts of fat (Malik *et al.*, 2013), thus various ingredients with low or zero caloric values have been developed as fat replacers, aiming to mimic the properties of fats in foods. These fat replacers can be based on lipids, carbohydrates or proteins. Fat replacers, although they often lack the physical and chemical properties of fats, can still replace certain functionalities of fats in foods under certain conditions.

Three categories of fat replacers have been suggested, based on different functionalities of fat in texture: 1) thickening agents to control flow properties, 2) bulking agents to enhance adsorption to the tongue, and 3) microparticulated ingredients to improve lubrication properties (Roller & Jones, 1996). Most fat replacers fall into the first two categories. Compared to the first two categories, the fat mimicking mechanisms of microparticulated fat replacers have not been subject to much research. Despite lack of this knowledge, the application of microparticulated fat replacers in several low fat food systems seems successful. Many microparticulated fat replacers are commercially available (Table I - 1). These ingredients exhibit a size range often comparable to that of homogenized fat globules (a few microns) in many types of processed foods. It is postulated that microparticulated particles in water-continuous food systems can be viewed to some extent as physically similar to oil droplets in an o/w emulsion (Roller *et al.*, 1996). The particles are assumed not detectable by the tongue (due to their micrometer size) (de Wijk *et al.*, 2005; Engelen *et al.*, 2005b; Tyle, 1993), yet spherical particles may nevertheless provide a lubrication effect due to a ball-bearing mechanism, where lubrication is important for the perception of creamy and smooth mouthfeel. In this thesis, both protein-based and carbohydrate-based microparticulated ingredients were investigated. Microparticulated protein was chosen as a spherical type of particle, and native micro-granular starch was chosen as a non-spherical type of particle.

### Microparticulated proteins

Microparticulated proteins derived from zein and egg white protein are both based on a protein-polysaccharide complex formation (i.e. zein with carboxymethylcellulose, and egg-white protein with xanthan). Microparticulated whey protein (MWP) is only based on whey protein concentrate from milk, and it is on this protein that we will focus in this thesis. Cheftel *et al.* (1993) report that MWP provides a smooth and creamy mouthfeel in low-fat foods. The biggest application of MWP so far has been in dairy based semi-solid and solid foods, including yoghurts (Tamime *et al.*, 1995; Torres *et al.*, 2009; Torres *et al.*, 2011),

ice creams (Singer & Dunn, 1990) and cheeses (McMahon *et al.*, 1996; Sahan *et al.*, 2008; Sturaro *et al.*, 2015). MWP has also been applied in non-dairy products, such as mayonnaise (Cheung *et al.*, 2002). So far, studies about MWP and its fat-replacing function have mostly focused on the effect it has on visual appearance (Chung *et al.*, 2014), viscosity (Cheung *et al.*, 2002; Chung *et al.*, 2014), microstructure and physical properties (Cheftel *et al.*, 1993; McMahon *et al.*, 1996; Schenkel *et al.*, 2013) as well as sensory properties (Cheung *et al.*, 2002; Torres *et al.*, 2011). There have been no studies of the tribological properties of MWP in different food matrices, even though the perception of fat-related sensory attributes has been hypothesized to be the consequence of ball-bearing lubrication (Cheftel *et al.*, 1993; Gaull, 1991).

**Table I - 1 Microparticulated ingredients as fat replacers (Roller *et al.*, 1996)**

Particle Type		Compositions	Commercial Examples
Protein based	Micro-particulated Proteins	Whey protein	Simplese® 100
		Egg white protein with whey protein and pectin	Simplese® 300
		Whey protein with hydrolyzed oat flour	Simplese® D-550
		Zein protein coated with polysaccharides	Lita®
Native starch	Starch from rice, quinoa, amaranth, parsnip, etc.		
Carbohydrate based	Modified starch	Maltodextrin from potato starch	Lycadex®; Paselli™
		Maltodextrin from corn starch	Novelose®; N-Lite™
		Acid hydrolyzed corn starch crystallites	Stellar™
		Maltodextrin from rice starch	RiceTrin®
	Cellulose	Colloidal microcrystalline cellulose (MCC)	Avicel®
Colloidal MCC & sodium carboxymethylcellulose		Tabulose SC	
Inulin	MCC co-processed with guar gum	Novagel™	
		Poly-fructose (DP = 2 - 60)	Fibruline®; Raftiline®

### Micro-granular starches

Starch is one of most commonly used ingredients for carbohydrate-based fat mimetics (Ognean *et al.*, 2006). In general, starch-based fat replacers were able to retain the texture of food by thickening, gelling, stabilizing substantial quantities of water, and melting (Alting *et al.*, 2009; Malinski *et al.*, 2003). Micro-granular starches might have the potential to combine the benefits of starch itself (which in general contributes to increasing viscosity and melting), with those of a microparticulated protein that improves lubrication. Microparticulated starches based on modified large granular starches (Setser & Racette, 1992) and native or modified micro-granular starches all exhibit particle sizes of a few micrometers. Nature

provides a wide range of micro-granular starches, such as parsnip (1 - 6  $\mu\text{m}$ ), rice (2 - 8  $\mu\text{m}$ ), amaranth (1 - 2  $\mu\text{m}$ ), cow cockle (0.3 - 1.5  $\mu\text{m}$ ), quinoa starches (0.5 - 3  $\mu\text{m}$ ), and fine fractions of wheat starches (2 - 10  $\mu\text{m}$ ) (Jane *et al.*, 1994; Lindeboom *et al.*, 2004; Wani *et al.*, 2012). These micro-granular starches are not all perfectly spherical, but largely polyhedral. By separating them from larger granules, one can obtain spherical micro-granular starch particles, e.g. small wheat starch particles (2 - 10  $\mu\text{m}$ ) in whole flour (Malinski *et al.*, 2003). Many of these micro-granular starches have been reported to act as natural fat replacers in foods, including ice creams (Chigurupati *et al.*, 1992; Mason *et al.*, 2009), frozen desserts (Malinski *et al.*, 2003), dressing (Bakal *et al.*, 1992), and sausages (Setser *et al.*, 1992). The “fat-like” mouthfeel caused by small micro-granular starches is typically attributed to small particle sizes (Joly & Anderstein, 2009), much like microparticulated protein. Malinski *et al.* (2003) suggested that these small starch granules might lubricate ice crystals and amplify creaminess in frozen desserts. However, there is a lack of research on lubrication properties of micro-granular starches. Zinoviadou *et al.* (2008) investigated tribological properties of several neutral polysaccharide solutions, including gelatinized cross-linked tapioca starches (with average particle size of 50  $\mu\text{m}$ ), which are much larger than the micro-granular starches that we are interested in. Only a few studies on tribological properties were reported even for other types of micro-particles in food, such as kappa carrageenan particles (Garrec *et al.*, 2013), whey protein aggregates (Chojnicka-Paszun *et al.*, 2014b; Chojnicka *et al.*, 2008), microcrystalline cellulose particles (Chojnicka-Paszun & de Jongh, 2014a), and chocolate particles (Lee *et al.*, 2004; Luengo *et al.*, 1997). In general, only limited information can be found regarding the lubrication properties of particle-containing foods.

## 1.7 Aim and outline of the thesis

The outline of this thesis is presented in Figure I - 4. The aim of this thesis is to understand the relationship between the structural, rheological and tribological properties during food breakdown, and the sensory perception of foods. More specifically, this thesis aims to link the properties of food particles in liquid and semi-solid matrices to the rheological and tribological properties in understanding the sensory perception of these systems. This thesis also aims to unveil the relationship between the molecular properties of food particles and their fat mimicking functionalities. Fat droplets and particle fat replacers based on protein and starch with micrometer size were investigated in this thesis. These particles were dispersed in liquid and semi-solid gel phases, forming the food model systems under consideration.



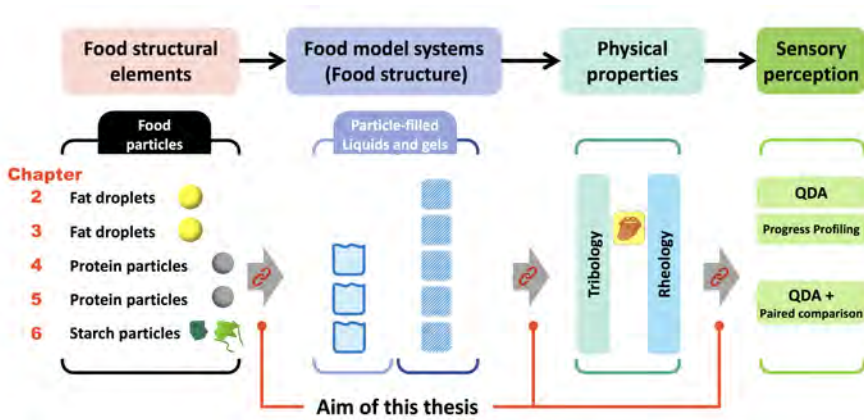


Figure 1 - 4 A schematic outline of the research treated in this thesis.

The aim of **chapter 2** is to understand the effect of fat droplet characteristics in emulsion-filled gels on their dynamic rheological, tribological and microstructure properties during breakdown, and their sensory perceptions. The fat droplet characteristics were varied by modulating the interaction of the fat droplets with the gelatin gel matrix, changing the fat droplet concentration and solid fat content in the droplets. A mouth-mimicking tribometer connected to CLSM was used to determine the friction and microstructural evolution of gels under shear. This tribometer was also employed in the subsequent chapters.

The emulsion-filled gels described in chapter 2 are further investigated in **chapter 3** to determine the influence of oral processing and fat droplet characteristics of emulsion-filled gels on the formation and clearance of fat deposition on the tongue in relation to sensory perception. In addition, the effect of follow-up consumption of liquid and semi-solid foods on the clearance dynamics of fat deposited on the tongue was investigated. The formation and clearance of fat deposited on the tongue was determined by *in vivo* fluorescence.

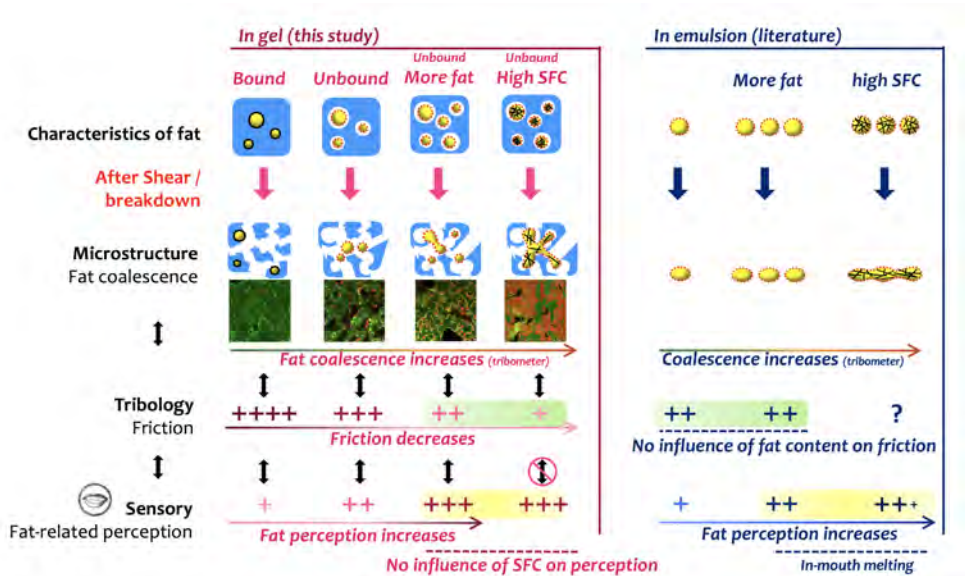
The purpose of **chapter 4** was to obtain a better understanding of the tribological properties of microparticulated whey protein (MWP) in liquid and semi-solid food model systems. Consequently, by investigating the sensory properties of MWP particles in relation to their rheological and tribological properties in these systems, **chapter 5** aims for a better understanding of the sensory perception of MWP particles compared to oil droplets in different food matrices. It also attempts to clarify the relationships between different sensory attributes that are often associated with the perception of particles.

In **chapter 6**, micro-granular rice starches are investigated as another potential particle fat replacer. Tribological properties of native and gelatinized rice starches in liquid o/w emulsions and semi-solid emulsion-filled gels were investigated. The aim of this chapter is to discuss the possible mechanisms underlying the fat mimetic properties of micro-granular particles.

The final chapter (**chapter 7**) summarizes the main findings of all the previous chapters and gives a general discussion about the underlying mechanisms of the tribological behaviors of different particle-filled food systems. Implications of the findings obtained from this thesis, along with recommendations for future work are also given.

# Chapter 2

## Fat droplet characteristics affect rheological, tribological and sensory properties of food gels



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## Abstract

This work aims to investigate the effect of fat droplet characteristics in emulsion-filled gels on their dynamic rheological, tribological and microstructure properties during breakdown, and their sensory perceptions.

Fat droplet characteristics investigated were the interaction of the fat droplet with the gel matrix (modulated by using different emulsifiers to yield droplets being either bound or unbound to the matrix) and the solid fat content (SFC, varying from 4% to 48%). Fat content was varied from 0% to 20%.

Elastic modulus and fracture properties of these gels (determined under uniaxial compression) were affected by droplet-matrix interaction, fat content, and SFC. A mouth-mimicking tribometer connected to a CLSM was used to determine tribological properties (friction) and microstructural evolution (fat coalescence) of gels under shear. Gels with unbound droplets led to more coalescence (than bound) and increased fat content also led to more coalescence. The observed increase in fat coalescence related to a decrease in friction, which was also related to an enhancement of the perception of fat-related sensory attributes (determined by quantitative descriptive sensory analysis).

The effects of unbound droplets and higher fat content on increasing coalescence and decreasing friction were further enhanced by increasing SFC. Having found that decrease in friction and increase in coalescence relates to an enhancement of perception of fat-related attributes, one would expect that increasing SFC would further enhance the perception of fat-related attributes. This was not found. We attribute this to the fact that our systems are gels that have complicated breakdown behavior.

## **2.1 Introduction**

Emulsion-filled gels as models for semi-solid and solid foods have been well studied because they allow to investigate the relationships between food structure and sensory perception (Dickinson, 2012). Emulsion-filled gels are model foods for a wide range of palatable products including yoghurt, cheese and processed meat products (Foegeding *et al.*, 2011). In these foods emulsified fats play an important role by providing an appealing texture and enhancing creamy mouthfeel (van Aken *et al.*, 2011). Overconsumption of fat, however, contributes to health problems such as overweight, obesity, and heart diseases (Drewnowski, 1997; van Aken *et al.*, 2011). Therefore, it is of great interest for the food industry to develop fat-reduced foods without compromising on texture and mouthfeel perception. The sensory perception of food is strongly influenced by its composition, interactions between its components and the dynamic oral processing (Dickinson, 2012; Hutchings *et al.*, 1988; Stokes *et al.*, 2013). In order to maintain or enhance the sensory appreciation of fat-reduced foods, a comprehensive understanding of the influence of fat properties on sensory perception during oral processing is needed.

The perception of food texture is regarded as a multidimensional sensory experience that is perceived during all phases of oral processing: initial phase (first bites), masticatory phase (chewing and bolus formation) and residual phase (swallowing and oral coating) (Brandt *et al.*, 1963; Chen, 2009; Stokes *et al.*, 2013). During each phase texture perception changes as the food structure and its properties constantly vary as a result of mechanical deformation and interaction with saliva (Chen *et al.*, 2012). Rheological and tribological properties at different oral processing phases have been demonstrated to strongly influence texture perception (Bellamy *et al.*, 2009; van Aken *et al.*, 2011). For emulsion-filled gels, the attributes firmness and brittleness are usually perceived in the first phase of oral processing. These attributes are primarily related to large deformation properties of foods (rheology dominant) (Brandt *et al.*, 1963). Mouthfeel attributes, such as chewiness, stickiness and creaminess, are often perceived in the masticatory phase (Brandt *et al.*, 1963). During this phase of oral processing the bolus is subjected to shear, flow, and displacement which are strongly influenced by both the rheological and tribological behavior of the bolus. In the last phase of oral processing the bolus is swallowed. Food residuals that adhere to oral surfaces are often sensed as creamy, smooth and velvety after-feel. After-feel perception is determined more by the tribological behavior of food residuals and their interactions with saliva (Prakash *et al.*, 2013). In summary, oral processing is dynamic and entails a “transition from rheology-dominant processes to tribology-dominant processes” (Stokes *et al.*, 2013).

Under conditions relevant for oral processing, tribology has been employed to study lubricational properties of foods and boli. Most studies on tribology focus on liquid (model) foods such as milk (Chojnicka-Paszun *et al.*, 2012), cream (Baier *et al.*, 2009; Kokini *et al.*, 1983), biopolymer solutions (Malone *et al.*, 2003) and emulsions (Dresselhuis *et al.*, 2008b; Dresselhuis *et al.*, 2008c; Malone *et al.*, 2003). It was found that coalescence of fat droplets occurring during oral processing led to a decrease of friction and to an increase of perception of fat-related sensory attributes, such as creaminess (Chojnicka-Paszun *et al.*, 2012; Dresselhuis *et al.*, 2008a; Dresselhuis *et al.*, 2008c; Dresselhuis *et al.*, 2007). The sensitivity of emulsion droplets towards coalescence under conditions mimicking oral processing behavior was found to change as a function of the stability of emulsions. The stability was modified by changing droplet size, emulsifier type and concentration, and solid fat content (SFC) (Dresselhuis *et al.*, 2008c).

Emulsions with higher SFC (palm fat) were found less stable than emulsions prepared with lower SFC (sunflower oil). The emulsions with higher SFC were also reported to coalesce more than emulsions with lower SFC under shear in a mouth-mimicking tribometer (Dresselhuis *et al.*, 2008c). This higher coalescence at higher SFC is presumably due to a higher sensitivity to rupture of the droplet interface upon deformation, where the rupture is caused by the presence of fat crystals penetrating the interface. The emulsions with higher SFC also related to higher fat-related perception (Benjamins *et al.*, 2009; Dresselhuis *et al.*, 2008c). These results suggest that SFC, coalescence of droplets in a tribometer and fat-related perception are related. However, the increased coalescence in a tribometer was not always found to relate to in-mouth coalescence (Dresselhuis *et al.*, 2008c). In addition, we think that instability of the emulsion droplets (against coalescence) is the most prominent reason for fat perception and that SFC is only one of the contributing factors. The effect of SFC can be overruled by other factors affecting the stability. For example, no influence of SFC is found when sodium caseinate is added as an extra emulsifier (Dresselhuis *et al.*, 2008c), and a less pronounced effect exists by adding not enough emulsifier (Dresselhuis *et al.*, 2008c) or by adding unsaturated mono-glycerides (Benjamins *et al.*, 2009).

Apart from liquid emulsions, semi-solid foods and solid foods are an important class of food products. As mentioned a suitable model system for these food products is an emulsion-filled gel. In contrast to the extensive studies on emulsions in relating microstructure with tribology and sensory, there are much less of these studies performed on emulsion-filled gels. Previous studies linked large deformation rheological properties of emulsion-filled gels to texture perception (Gwartney *et al.*, 2004; K.-H. Kim *et al.*, 1996; Sala *et al.*, 2008). Several experimental techniques that combine rheological and tribological measurements

with observations of the microstructure of the food under mechanical deformation have been developed and applied to study composite gels during breakdown and mastication (Abhyankar *et al.*, 2011; Nicolas & Paques, 2003; Nicolas, Paques, *et al.*, 2003). It has been demonstrated for emulsion-filled gels that creaminess relates to the type of gel matrix and the oil content. It was also found to relate to the release of oil droplets from the gel matrix during gel breakdown when squeezed out of a syringe (resembling in-mouth conditions) (Chojnicka *et al.*, 2009; Sala *et al.*, 2007b). Release of oil droplets from the gel matrix during mechanical breakdown could be enhanced when oil droplets were unbound to the matrix (i.e. not interacting with the gel matrix) (Chojnicka *et al.*, 2009; Sala *et al.*, 2007b).

In summary, in liquid emulsions fat-related perception can be enhanced by increasing the instability of droplets against coalescence, and in emulsion-filled gels fat-related perception can be enhanced by increase of oil release. We hypothesize that the enhancement of fat-related perception due to increase of oil release can be further enhanced by increasing the instability of emulsion droplets against coalescence. We have tested this hypothesis in emulsion-filled gels where the droplets are bound to the gel matrix, and in emulsion-filled gels where the droplets are unbound. In the current work we have chosen to vary the droplet instability by varying the SFC.

According to the hypothesis we have three expectations. Expectation A is that we expect more droplet release and coalescence under shear in a tribometer by incorporating fat droplets with higher instability towards coalescence (higher SFC) into emulsion-filled gels with high droplet releasing ability (unbound). Expectation B is that the increased coalescence of droplets will give rise to formation of a lubricating fat layer between the shearing surfaces of a tribometer, decreasing friction. This increased coalescence and the formed fat layer is expected to be observable under a microscope. Expectation C is that the above leads to an enhanced fat-related perception. This expectation is based on the assumption that increase of coalescence and formation of fat layer in a tribometer also happens in mouth in a similar way during oral processing. Taking into the account that tribology and sensory are two main aspects of testing our hypothesis we have treated these aspects in two sections of our paper.

Because of the fact that oral processing entails a “transition from rheology-dominant processes to tribology-dominant processes” (Stokes *et al.*, 2013) we also have to consider a third aspect in our study: bulk rheology. Indeed, large deformation rheological properties of semi-solid foods can often be related to their texture perceptions. For example, Young’s modulus relates to firmness perception (Kramer *et al.*, 1973). It is expected that by changing the SFC in droplets, and by changing the droplet-matrix interaction, the large deformation properties of the gels would be

influenced (Oliver *et al.*, 2015; Sala *et al.*, 2007a). We note that rheology-related perceptions may interrelate with tribology-related perceptions. In order to interpret observed effects of SFC on sensory perception being independent from gel rheology we studied the sensory properties with and without controlling the rheological properties. Because of this importance of the rheological properties we have assembled the according results in a separate section.

In short, the aim of this study was to determine and understand the combined effect of instability of fat droplets towards coalescence (by varying in SFC) and droplet releasing ability of the gel (by varying droplet-matrix interaction) on the large deformation rheological properties, tribological properties, microstructure, and sensory properties of emulsion-filled gels. This study also aims to contribute to understand the inter-relationships among these properties of emulsion-filled gels. The findings from this study should provide useful information for designing fat-reduced solid or semi-solid foods with required texture and sensory properties.

## 2.2 Materials & Methods

### 2.2.1 Materials

Beef fat (Sonac edible tallow max 1), hard pork fat (Sonac edible lard max 1), and poultry fat (Sonac Edible Poultry Fat) were provided by Sonac (Son, the Netherlands). Soft pork fat was obtained from Ten Kate Vetten B.V. (Ter Apelkanaal, the Netherlands). Mixed pork fat was prepared by mixing hard and soft pork fat (mixing ratio 1:1, w/w). Medium Chain Triglycerides (MCT) oil was obtained from Internatio BN (Mechelen, Belgium). Porcine skin gelatin (bloom value 240-260) was provided by Rousselot (Gent, Belgium). Powdered whey protein isolate (WPI, Bipro™) was obtained from Davisco International Inc. (La Sueur, MN, USA). Tween 20 (Polyoxyethylene sorbitan monolaurate) and paraffin oil were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sweetener (Natrena, main components: cyclamate, saccharin and acesulfame-K) and vanilla flavor (Dr. Oetker) were bought from a local supermarket (Ede, the Netherlands). Titanium dioxide (TiO<sub>2</sub>, Ppretiox®) was obtained from (Preceza, Prerov, Czech Republic). All materials were used without further purification. All samples for physical measurements were prepared with Reverse Osmosis water (RO water). All sensory samples were prepared with regular tap water.



## **2.2.2 Preparation of Emulsions**

Oil-in-water emulsions of 40% fat or oil were prepared with different emulsifiers. Emulsions were stabilized either with 1% (w/w) whey protein isolate (WPI) or 2% (w/w) Tween 20 in the aqueous phase. The 1% (w/w) WPI solution was made by dissolving and stirring the powdered WPI in water at room temperature for 1.5 hrs. The 2% (w/w) Tween 20 solution was prepared similarly. Hard pork fat and beef fat were melted in a water bath at 60°C before homogenization. Soft pork fat, poultry fat and mixed pork fat were melted in a water bath at 45°C before homogenization. MCT oil was used at room temperature. Oil-in-water emulsions (40%, w/w) were prepared by mixing the fat or oil with the emulsifier solutions. Emulsifier solutions were pre-heated to the temperature of corresponding fat or oil before mixing. After pre-homogenization with an Ultra Turrax Polytron (Kinematica AG, Switzerland) for 2 min, a lab homogenizer Ariete (NS1001L-Panda, Niro Soavia, Parma, Italy) was used at homogenization pressures ranging from 180 bar to 310 bar to produce the emulsions with a comparable droplet size. The lab homogenizer was pre-heated by passing through hot water for 3 min before homogenization.

The droplet size distributions of all emulsions were measured with a MasterSizer2000 (Malvern Instruments Ltd., Malvern, UK). Volume-to-surface diameter ( $d_{3,2}$ ) was used as the mean droplet size. The average diameter of the fat droplets and oil droplets was  $1.5 \pm 0.2 \mu\text{m}$ . The calculation of droplet size distribution was based on a uni-model distribution with fitting accuracy above 99%.

The solid fat content (SFC) at 20°C of fat droplets was determined for all 40% (w/w) emulsions using a pulsed nuclear magnetic resonance (p-NMR) device (Minispec MQ20, Bruker, Karlsruhe, Germany). The complete SFC profile of each fat in the temperature range from 0°C to 60°C is shown in Appendix II - C (Figure II - C. 1 and Figure II - C. 2). Emulsions were filled into NMR tubes to a height of 15 mm, and then were stored in a refrigerator at 5°C for 20 hrs to allow fat crystallization. The SFC was measured according to the method described by Gribnau (Gribnau, 1992).

## **2.2.3 Preparation of emulsion-filled gels**

The previously prepared 40% (w/w) emulsions stabilized with WPI or Tween 20 were mixed with gelatin solutions at different ratios. The final concentration of gelatin in the aqueous phase of the gel was 4% (w/w). The final concentration of fat in the whole gel ranged from 0% to 20% (w/w). Gelatin was first dissolved in water and hydrated under gentle stirring for 2 hrs at room temperature. The gelatin solutions were heated at 80°C for 30 min in a water

bath and then cooled to 25°C by flowing tap water. Sweetener mixture (1.5 - 2.5%, w/w) and vanilla flavor (1 - 3%, w/w) were added to the gel solutions for all samples used for sensory testing. TiO<sub>2</sub> (0.35%, w/w) were added to gels X and Y to make the gels opaque. The mixtures were immediately stored in the refrigerator at 5°C. Gels were stored at 5°C for 20 hrs and then kept at 20°C for 2 hrs before characterization.

The emulsion-filled gels used for large deformation measurements were allowed to gel in 60 ml plastic syringes (internal diameter 26.4 mm) coated with a thin layer of paraffin oil. The gels were removed from the big opening of syringes by being air-pushed from the small opening. The small opening was connected to another syringe filled with air via a rubber tube. The gels for tribological measurements were prepared in 30 ml plastic syringes (internal diameter 21.3 mm) without coating a paraffin oil layer. The gels for sensory evaluations were prepared under food-grade conditions and were gelled in 30 ml transparent, plastic cups sealed with plastic lids. An overview of the composition of emulsion-filled gels used for sensory evaluations is given in Table II - 1.

**Table II - 1 Composition of sensory samples**

<b>Sample Name</b>	<b>Droplet-matrix interaction</b>	<b>Fat (% w/w)</b>	<b>Fat type</b>	<b>Gelatin (% w/w)</b>
A	Bound	5	Soft pork fat (SFC=4.0%)	4
B	Bound	5	Mixed pork fat (SFC=18.5%)	4
C	Bound	5	Hard pork fat (SFC=35.7%)	4
D	Unbound	5	Soft pork fat	4
E	Unbound	5	Mixed pork fat	4
F	Unbound	5	Hard pork fat	4
G	Unbound	15	Soft pork fat	4
H	Unbound	15	Mixed pork fat	4
I	Unbound	15	Hard pork fat	4
J	Unbound	5	Soft pork fat	5.25
K	Unbound	5	Mixed pork fat	5.15
L	Unbound	5	Hard pork fat	5.05
M	Unbound	15	Soft pork fat	5.5
N	Unbound	15	Mixed pork fat	5.25
O	Unbound	15	Hard pork fat	5
X	-	0	-	4
Y	-	0	-	5

#### **2.2.4 Characterization of large deformation properties**

An Instron universal testing machine (M5543, Instron International Ltd., Belgium) equipped with plate-plate geometry was used to perform uni-axial compression tests on emulsion-filled gels. The cylindrical gel specimen were 25 mm high, and the diameter of gel specimen was 26.4mm. Both the plate and the top of the gel surface were lubricated with a thin layer of paraffin oil to prevent friction between plate and sample during compression. To determine fracture behavior, all measurements were performed at 20°C at a constant compression speed of 1 mm/s up to a compression strain of 80%. To determine recoverable energy, the measurements were performed at 20°C at a constant compression and decompression speed of 1 mm/s up to a compression strain of 20% (van den Berg *et al.*, 2008). The ratio between the energy release during the decompression and the energy needed to compress the gels is defined as the recoverable energy. All measurements were conducted eight times and the values were averaged.

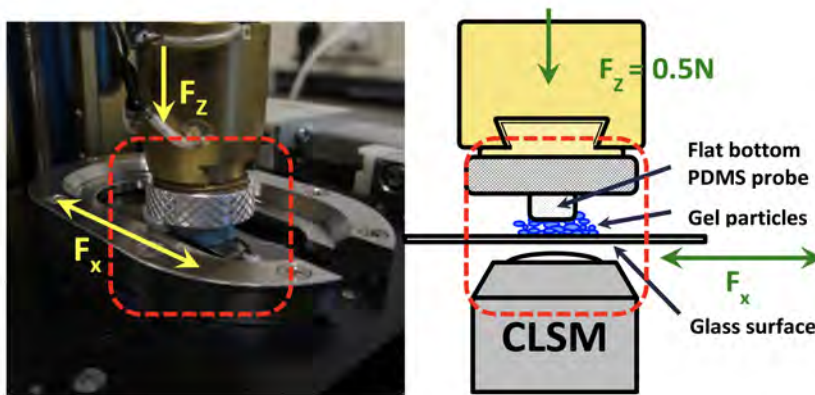
#### **2.2.5 Confocal Laser Scanning Microscopy (CLSM)**

Micro-structural analysis of emulsion-filled gels used for large deformation compression test were stained with 0.2% (w/w) Rhodamine B to visualize the protein phase. Rhodamine B was added to the sample surface prior to CLSM measurement. Micro-structural analysis of emulsion-filled gels used for tribological measurements were stained with 0.5% (w/w) Nile blue solution to visualize the fat phase. Nile blue was added to the gel samples during the sample preparation. The final concentration of Nile blue in the gel was 0.005% (w/w). CLSM images were recorded on a LEICA TCS SP5 Confocal Laser Scanning microscope (Leica Microsystems CMS GmbH, Mannheim, Germany) equipped with an inverted microscope (Leica DM IRBE). An Ar/Kr visible light laser was used when samples were dyed with Rhodamine B, and an Argon-HeNe633 laser was used when samples were dyed with Nile blue. The objective lens used was HCX PL APO 63x/1.20 Water CORR CS (Leica). Digital images were acquired at a resolution of 1024x1024 pixels.

#### **2.2.6 Optical Tribological Configuration (OTC)**

The tribological properties and the microstructure of the gels under deformation were determined with a self-built Optical Tribological Configuration (OTC)(Figure II - 1) (Dresselhuys *et al.*, 2007)). A certain amount of emulsions (200 µl) or gels (around 200 mg) were sheared in the OTC between two surfaces. The upper surface is a flat-bottom rough PDMS probe (Sylgard 184 Dow Corning, USA; mixing ratio of PDMS : cross linker = 10:1; diameter 6mm)

and the lower surface is made of glass (Dresselhuis *et al.*, 2008b). The load ( $F_z$ ) between the upper surface and sample is 0.5 N. During each measurement the lower glass plate was oscillating 10 cycles over a distance of 16 mm at an increasing oscillating speed from 10 to 80 mm/s, with incremental steps of 10 mm/s. During the movement of the glass plate, the friction force ( $F_x$ ) was determined. Each measurement was performed at 20°C with a new probe. The surfaces of probe and glass were cleaned with ethanol and water before each measurement. All measurements were conducted in triplicate. The microstructure of the gel before performing tribological experiments was observed with CLSM after the sample being compressed by the PDMS probe, and the microstructure after tribological experiments was observed with CLSM immediately after the glass plate stopped moving.



**Figure II - 1 Optical Tribological Configuration:** Emulsions or gel pieces are sheared between an upper PDMS surface and a lower glass plate. A force ( $F_z$ ) is applied and the friction force ( $F_x$ ) measured while the glass surface oscillates at a given speed. A Confocal Laser Scanning Microscopy (CLSM) is placed under the moving glass plate to observe the microstructure of the sample during deformation.

Prior to the OTC tribological measurements, gels were pre-treated by being disrupted into smaller pieces to mimic chewed foods under oral conditions. The gels were squeezed through the orifice of a syringe connected with a 200 $\mu$ l pipette tip. This squeezing was performed by moving the syringe plunger at a constant speed (1 mm/s) for 40 s with an Instron (Sala *et al.*, 2007b). During the squeezing the syringe was fixed in a vertical position by several racks. The 5cm long pipette tip has a cone shape with an opening of 0.2 mm internal diameter. The broken-down gel pieces were collected and analyzed by CLSM and OTC.

### **2.2.7 Quantitative descriptive analysis (QDA) of sensory properties of emulsion-filled gels**

The sensory properties of 17 emulsion-filled gels (Table II - 1) were evaluated by a trained sensory panel (n = 11) according to the principles of Quantitative Descriptive Analysis (QDA) (Stone & Sidel, 2004). All panelists were females and aged between 43 and 65 years. All panelists were experienced in QDA profiling of semi-solid food gels and o/w emulsions from several previous studies. For this study, all panelists were trained in the description of odor, flavor, taste, mouthfeel and after-feel attributes of the emulsion-filled gels. Six training sessions of 2 hrs were conducted. Among these six sessions, four training sessions were used to generate the attributes. After attributes generation, two training sessions were used to define the attributes and to define the instructions to evaluate these attributes. In total 25 attributes that were generated by the panelists were used in the data analysis (Table II - 2). The attributes were generated in Dutch and then were translated into English. After the training, four 2-hour profiling sessions were conducted in sensory booths at 20°C with appropriate ventilation and lighting. All samples were evaluated in duplicate by the n=11 panelists during separate sessions (more details can be found in Appendix Table II - A. 1). Samples were presented in randomized order and labelled with three-digit codes. In each session, twelve samples were presented one after another to the panelists, of which one was a “warm-up” sample and two were reference samples without fat. The warm-up sample was the same as one of the samples (randomly chosen), and it was presented first in order to familiarize the panelists with the taste of the samples. The reference samples were presented in random order (together with all other samples). Each gel was served in a portion of around 20g in a plastic cup (volume 25ml). Each sample was first smelled to have the odor attributes rated. Then the panel took the samples in mouth using a spoon and evaluated the odor, mouthfeel and taste attributes in the order that they were perceived. Afterwards the samples were spat out and after-feel and after-taste were evaluated. Between two different samples, the panelist cleaned their mouth with sparkling water and crackers, and tap water could be taken ad libitum.

Acquisition of the responses from the panel was collected using FIZZ software (Biosystemes, V2.41b, 2009). The panel performance check was done with Senpaq (V3.2, 2007). Univariate analysis of variance (UNI-ANOVA) (IBM SPSS, V20.0.0) using a general linear model (GLM) was performed to analyze the effect of sample type on individual sensory attributes. Fixed factor was sample type. Random factors of panelists and duplicate session were also included. Tukey's honest significant difference (Tukey's HSD) test was performed as a post analysis. In this test a level of significance of  $p < 0.05$  was chosen. Principal component analysis (PCA)

(Biosystemes, V2.41b) was used to analyze the relationships between sensory attributes and physical characteristics of the emulsion-filled gels. Pearson linear correlation coefficient (R2) was calculated (STATISTICA, V10, StatSoft) to analyze the relationships between each two sensory attributes and relationships between sensory attributes and large deformation properties of the samples.

**Table II - 2 Description of sensory attributes given by QDA panel**

	Description given by the QDA panel
<b>Odor attributes</b>	
O-intensity	Total quantity of odor
O-vanilla	Chemically, as artificial flavoring
<b>Mouthfeel attributes</b>	
*MF-firm	Effort needed press (first impression)
*MF-elastic	Resilient when pressed, like a rubber band
*MF-brittle	How quick the product breaks when crushed
*MF-pieces	Falling apart in pieces
*MF-spreadable	Like a paste
MF-dry	Dry feeling in the mouth
*MF-watery	Becomes a liquid
*MF-melting	Dissolves in the mouth, the structure disappears like in ice cream
*MF-fatty	Oil-like
*MF-sticky	Clings in the mouth and on the lips
*MF-slimy	mucus
*MF-slippery	Slithering
*MF-creamy	Full, soft, velvety feeling, total impression
MF-pungent	Pepper sensation
MF-astringent	Tart, like from unripe apple, medlar and quince
<b>Taste attributes</b>	
T-intensity	Total quantity of taste
T-mild	A soft taste
T-sweet	Basic taste and of artificial sweetener
T-bitter	Basic taste
<b>After-feel attributes</b>	
*AF-fatty	Oil-like
*AF-coating	A layer covering the mouth
<b>After-taste attributes</b>	
AT-sweet	Basic taste and of artificial sweetener
AT-bitter	Basic taste

**\*Mouthfeel and after-feel attributes that are related to texture perception and fat sensations are marked with an asterisk.**

## 2.3 Results and discussion

### 2.3.1 Solid Fat Content

Table II - 3 shows the Solid Fat Content (SFC) of 40% o/w emulsions at 20°C. The SFC of emulsions used in this research ranges from 4.0% to 47.5%.

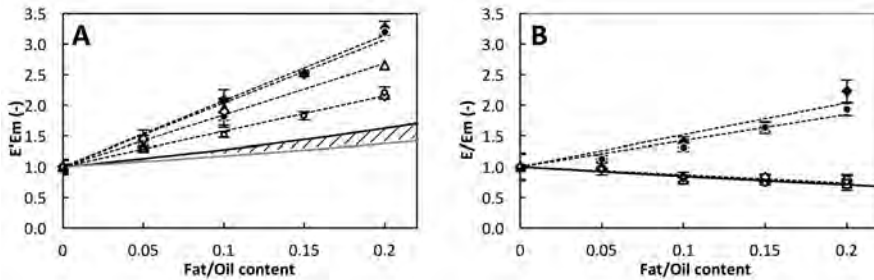
**Table II - 3 Solid Fat Content (SFC) of 40% o/w emulsions at 20°C. Mixed pork fat is a mixture of soft pork fat and hard pork fat at ration 1:1 (w/w).**

Fat in emulsion	Solid Fat Content (%)
MCT oil	4.4
Soft pork fat	4.0
Poultry fat	9.3
Mixed pork fat	18.5
Hard pork fat	35.7
Beef fat	47.5

### 2.3.2 Large deformation properties and initial microstructure of emulsion-filled gels

Young's modulus  $E$  of emulsion-filled gels varying in SFC as a function of fat content is shown for bound droplets (Figure II - 2A) and unbound droplets (Figure II - 2B). The Young's modulus of the emulsion-filled gels  $E$  were normalized by the Young's modulus of the gelatin gels without emulsions  $E_m$  (0% fat). No emulsifier was added to the gelatin gels without emulsions (Sala *et al.*, 2007a).

Figure II - 2A shows  $E/E_m$  of emulsion-filled gels with bound droplets (WPI stabilized) together with the predicted value calculated with van der Poel's theory for bound fillers. The theory was developed to calculate the shear modulus of highly concentrated spherical particle dispersion (van der Poel, 1958). The theory was simplified by Smith (Smith, 1975) and was also applied to particle-filled gels (van Vliet, 1988). Van der Poel's theory allows to calculate the shear modulus of particle containing composite gels in which the particles are bound to the matrix (Figure II - 2A). For particles that are unbound, the effective filler modulus is assumed to be zero, according to the modification on Van der Poel's theory as proposed by van Vliet (van Vliet, 1988). The normalized Young's modulus  $E/E_m$ , which is the same as the ratio between the shear modulus of the gel and gel matrix, is calculated. Only predictions of beef fat filled gels and MCT oil filled gels are shown in Figure II - 2A and B, as they represent the fats and oils with the highest and lowest SFC values.  $E/E_m$  of filled gels containing hard pork fat, poultry fat and soft pork fat are expected to fall between the two predictions.



**Figure II - 2** Normalized Young's modulus of emulsion-filled gelatin gels: A: fat droplets stabilized with WPI (bound); B: fat droplets stabilized with Tween 20 (unbound) (◆ beef fat, ● hard pork fat, △ poultry fat, ◇ soft pork fat, ○ MCT oil; grey line: prediction based on van der Poel's theory for MCT oil filled gel; black line: prediction based on van der Poel's theory for beef fat emulsion-filled gel; dotted lines are shown to guide eyes).

In Figure II - 2A Young's modulus increases with increasing fat content for all types of fat indicating that fat droplets are bound. The fat droplets participate in building up the matrix structure through the interaction between emulsifier (WPI) and matrix (gelatin). Similar observations were found by other researchers as well (Oliver *et al.*, 2015; Sala *et al.*, 2007a). With increasing SFC (filler modulus), the increase of Young's modulus with fat content also increased. This is in agreement with van der Poel's theory that predicts an increasing gel modulus with increasing filler modulus. We observe that the increase in Young's modulus with increasing fat content is larger than predicted by van der Poel's theory, and becomes larger with increasing SFC. The deviation between experimental data and theory might be caused by fat droplets forming aggregates that enclose the gel matrix during gelation in the emulsion-filled gels (Sala *et al.*, 2007a). For our experimental conditions, the gelation takes around 30min, which might allow some fat droplets to form larger clusters due to van der Waals attractions, hydrophobic and depletion interactions (Sala *et al.*, 2007a). It is also possible that during homogenization part of the fat droplets aggregate due to shear induced aggregation (Chen *et al.*, 1998). As a consequence of fat droplet aggregation, the effective volume fraction of droplet aggregates is larger than the total volume of all contributing individual droplets (Sala *et al.*, 2007a; van Vliet, 1988). In our study a good fit of the experimental data to van der Poel's theory is obtained when  $\phi_{\text{effective}}/\phi_{\text{filler}}$  is 2.5, which is consistent with literature (Chen *et al.*, 1998; van Vliet, 1988). Furthermore, the anisometry and homogeneity of the droplet aggregates may also have influence on the rheological properties of the filled gels (Nardin & Papirer, 2006).

The microstructure of the emulsion-filled gels containing MCT oil and beef fat droplets stabilized with WPI are shown in Figure II - 3. Qualitatively, droplet aggregation is observed



to a limited extent, especially in emulsion-filled gels with higher fat/oil content. Beef fat droplets appear to aggregate more than MCT oil droplets, which explains the stronger increase in normalized Young's modulus of the beef fat emulsion-filled gels compared with the MCT oil droplet filled gels. The deviation between van der Poel's theory and our experimental data could also be ascribed to the fact that in van der Poel's theory the fillers are non-deformable, evenly distributed, spherical particles with no interactions with each other (van der Poel, 1958). In contrast, the emulsion droplets used as fillers in the gels studied here are deformable and partly aggregated.

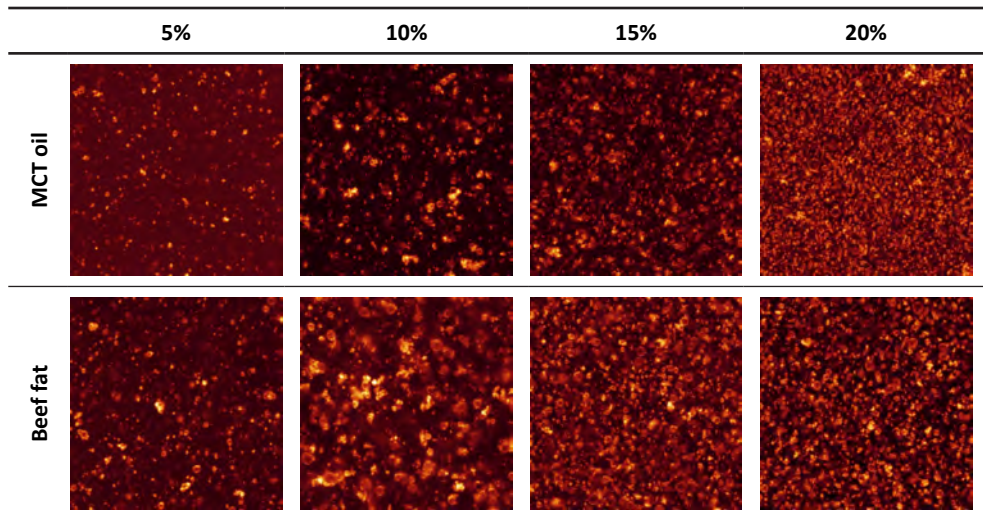
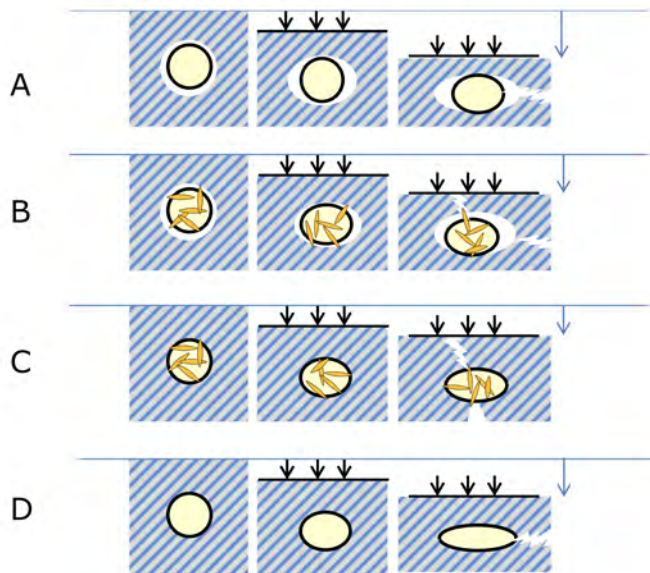


Figure II - 3 Microstructure of emulsion-filled gels: CLSM images of 4% (w/w) gelatin gels containing MCT oil droplets and beef fat droplets (varying from 5% to 20%, w/w) stabilized with 1% (w/w) WPI. Bright areas represent fat. The image size is 68.2  $\mu\text{m}$  x 68.2  $\mu\text{m}$ .

Figure II - 2B shows E/Em of emulsion-filled gels with unbound droplets (Tween 20) together with the predicted values calculated with van der Poel's theory for unbound fillers (filler modulus=0). Tween 20 is a non-ionic surfactant which has little physical or chemical affinity with the gelatin matrix, so the fat droplets are expected to be unbound to the matrix (Dickinson & Chen, 1999). For fat droplets with relative low SFC (<10%, soft pork fat, poultry fat and MCT oil), Young's modulus E/Em decreases with increasing fat content and is well predicted by van der Poel's theory. This is in good agreement with previous studies (Sala *et al.*, 2007a). Unbound droplets or inactive fillers are assumed not to strengthen the gel matrix, and the modulus of the composite gel decreases with increasing droplet content. This is because small deformations of the gel matrix are assumed not to deform the unbound droplets, but only the intermediate aqueous layer between droplets and matrix (Figure II -

4A) (van Vliet, 1988; Yang *et al.*, 2011). Because the aqueous layer creates structural defects within the matrix they act as “structure breakers” (Chen & Dickinson, 1999; Yang *et al.*, 2011). For unbound fat droplets with relative high SFC (>36%, beef fat and hard pork fat), an increase of Young’s modulus  $E/E_m$  with increasing fat content is observed. This might be caused by fat crystals inside the droplets participating in the matrix network and reinforcing the gel. Nevertheless, the intermediate aqueous gap between unbound droplets and matrix exists in gels filled with high SFC fats. Gels with unbound droplets cannot be strengthened by fat crystals as much as gels with bound droplets. This might explain why the Young’s moduli of gels with unbound beef and hard pork fat droplets (Figure II - 2B) are lower than the bound ones (Figure II - 2A).



**Figure II - 4** Schematic representation of assumed structural evolution of emulsion-filled gels under compression. A: unbound fat droplets, low SFC; B: unbound fat droplets, high SFC; C: bound fat droplets, high SFC; D: bound fat droplets, low SFC. The dark yellow anisotropic particles represent fat crystals. The white irregularities within the gel matrix represent cracks.

The normalized fracture strain and stress of emulsion-filled gels varying in SFC and interactions between fat droplets and gelatin matrix (bound/unbound) as a function of fat content are shown in Figure II - 5 and Figure II - 6.

In Figure II - 5A (droplets bound to the matrix), increase of fat content leads to a decrease in

fracture strain for droplets containing different amount of solid fat. The extent of decrease depends on the SFC in the droplets. It is clear that droplets with relatively high SFC (beef fat and hard pork fat) induce a faster drop in fracture strain of the gels. One possible explanation is that the high amount of fat crystals might produce more structural defects during the formation and deformation of gels (Figure II - 4C, D). Furthermore, the inhomogeneous aggregation of fat droplets that we discussed before might behave like stress concentrators which quickly initiate larger defects in the gel that causes fracture, therefore decrease the fracture strain (Ajayan *et al.*, 2003).

In Figure II - 5B (droplets unbound to the matrix), the effect of fat content on the fracture strain does not show a trend. The fluctuation of fracture strain data is probably due to the different extent of aggregation in different samples. In general, droplets with relative higher SFC (beef fat and hard pork fat) induce decrease in fracture strain, whereas droplets with lower SFC induce increase in fracture strain at low fat content. Other than the influence of aggregates and fat crystals (droplets with lower SFC are more deformable), fracture could happen at a higher strain than the less deformable droplets (with higher SFC) when droplets are unbound. By comparing Figure II - 5A and B, we observe that gels with unbound droplets have higher fracture strain. Indeed bound droplets are reported to induce a lower fracture strain (Nielsen, 1966). This is attributed to a higher stress at the surface of the bound droplets than unbound droplets. With the unbound droplets the water layer dissipates part of this stress.

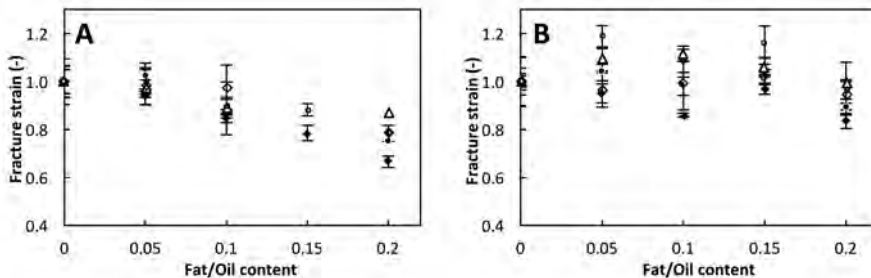
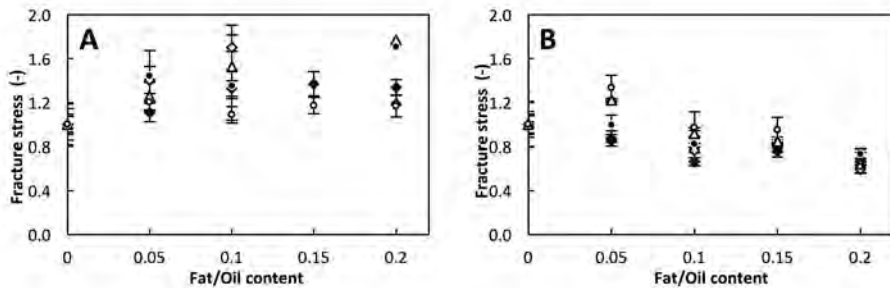


Figure II - 5 Normalized fracture strain of emulsion-filled gelatin gels: A: stabilized with WPI (bound); B: stabilized with Tween 20 (unbound) (◆ beef fat, ● hard pork fat, △ poultry fat, ◇ soft pork fat, ○ MCT oil). Fracture strain of emulsion-filled gels were normalized by the fracture strain of the gelatin gel without emulsion (0% fat, w/w).

In Figure II - 6A (droplets bound to the matrix), increase of fat content seems to induce an increase of fracture stress for droplets with high SFC (beef fat and hard pork fat). The increase of fracture stress can be attributed to the increasing amount of fat crystals in the

droplets and may also be affected by an increasing amount of droplet aggregates enclosing part of the gel matrix that strengthen the gels.

In Figure II - 6B (droplets unbound to the matrix), increase of fat content to 20% yields a slight decrease in fracture stress. The influence of SFC is different at different fat/oil content. This may be attributed to several effects that may partially compensate one another to an extent that is different for different fat/oil content. These effects may for example be that higher SFC yields a higher Young's modulus and a lower fracture strain, and droplet aggregation.



**Figure II - 6 Normalized fracture stress of fat emulsion-filled gelatin gels: A: stabilized with WPI (bound); B: stabilized with Tween 20 (unbound) (◆ beef fat, ● pork fat, △ poultry fat, ◇ soft pork fat, ○ MCT oil). Fracture stress of emulsion-filled gels were normalized by the fracture stress of the gelatin gel without emulsion (0% fat, w/w).**

To describe the fracture behavior of gels containing particle fillers, many theories and modifications on these theories are developed (Langley & Green, 1989; Nielsen, 1966). However, all theories mentioned above failed to accurately predict the effect of fillers on fracture stress or strain of emulsion-filled gels (Sala *et al.*, 2007a). This can be ascribed to the fact that many conditions of the current work are different from the theoretical assumptions, such as the size, homogeneity, rigidity and hydrophobicity of fillers, the existence of aggregations, the amount of fat crystals inside fillers, and the complex interactions between fillers and matrix in the large deformation regime.

Figure II - 7 shows the recoverable energy of emulsion-filled gels containing droplets of different fat types with accordingly different SFC as a function of droplet fraction. When droplets are bound to the matrix (Figure II - 7A), neither fat content nor SFC had a considerable influence on the recoverable energy of gels. Only droplets with highest SFC (beef fat) show a very small decrease in recoverable energy at high fat contents. When droplets are unbound to the matrix (Figure II - 7B), an increase of fat content leads to a slight decrease in recoverable energy (i.e. more micron scale fracture events in the gel matrix)

when SFC is relatively low (SFC<10%). The decrease in recoverable energy with increasing fat content becomes more pronounced when SFC is high (SFC>36%).

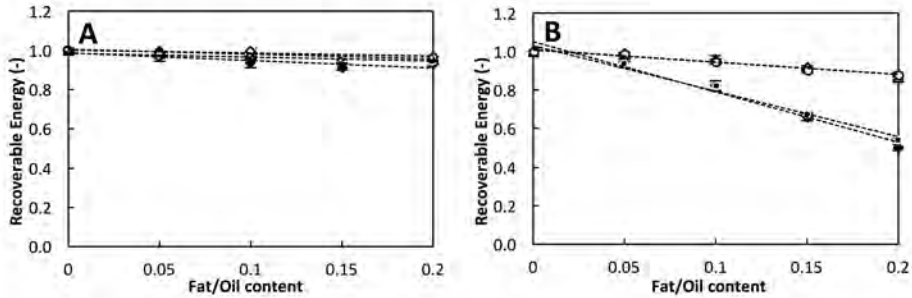


Figure II - 7 Normalized recoverable energy (at 20% compression) of fat emulsion-filled gelatin gels: A: stabilized with WPI (bound); B: stabilized with Tween 20 (unbound) (◆ beef fat, ● hard pork fat, △ poultry fat, ◇ soft pork fat, ○ MCT oil). Recoverable energy of emulsion-filled gels were normalized by the recoverable energy of the gelatin gel without emulsion (0% fat, w/w). Dotted lines are shown to guide eyes.

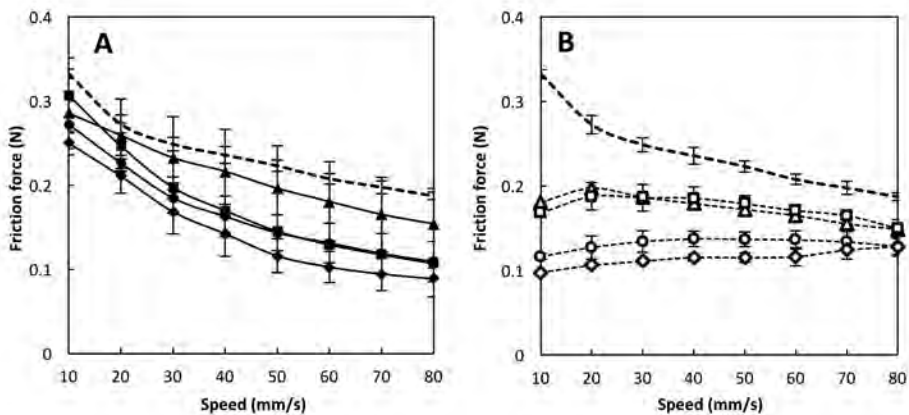
Gelatin is known as a very elastic material that can store considerable amount of energy during deformation before fracturing. Bound droplets have fixed position within the gel matrix and participate in the gelatin network. We expect an affine deformation of the droplets with the gelatin network when mechanical stress is applied. Therefore, gels with bound droplets store most of the energy during deformation. The slight decrease in recoverable energy for gels with beef fat droplets (cf. Figure II - 7A) can be attributed to a slightly higher energy dissipation within the fat droplets (being highest for the hardest fat, i.e. highest viscosity). In the unbound droplet case we observe (cf. Figure II - 7B) a more pronounced decrease of RE as a function of fat/oil content because of the presence of an aqueous layer around them, leading to higher viscous dissipation (van de Velde *et al.*, 2011). This effect apparently is much larger than the effect of increased viscosity with the droplets at increased SFC. This explains why gels with unbound fat droplets with high SFC show a stronger decrease in recoverable energy (i.e. more dissipation) upon increasing fat content. When SFC is increased, the concentration increase in viscosity of the fat phase leads to larger dissipation.

### 2.3.3 Tribological properties and according microstructural evolution of emulsion-filled gels

Figure II - 8 shows the friction force of pre-sheared emulsion-filled gels containing droplets with high SFC. When droplets are bound to the gel matrix (Figure II - 8A), friction force

decreases by up to 65% with increasing moving speed from 10 to 80 mm/s. With increasing fat content from 5% to 20%, friction decreases by about 20% at low speed (10-20 mm/s), and decreases by about 40% at high speed (80 mm/s). Gelatin gels without emulsion droplets also show a decrease in friction force with increasing speed. The friction force of gelatin gels without emulsion droplets is higher than any gel with emulsion droplets.

When droplets are unbound to the matrix (Figure II - 8B), the friction at low fat content (5% and 10%) did not change considerably with speed. A small increase in friction with speed is observed at higher fat content (15% and 20%). With increasing fat content from 5%-20%, friction decreased by about 45% at low speed (10-20 mm/s) and by about 15% at high speed (80 mm/s). At low speed (10 – 40 mm/s), friction of gels with unbound droplets are lower than bound ones; whereas at higher speed (> 50mm/s), friction of gels with unbound droplets are similar to or slightly higher than bound ones.



**Figure II - 8** Friction force of pre-treated emulsion-filled gels containing hard pork fat (SFC=36%) as a function of speed. Filled symbols represent gels with bound droplets (A), empty symbol represent gels with unbound droplets (B). Fat content: ▲△ =5%; ■□ =10%; ●○ =15%; ◆◇ =20%. Discontinuous line without symbols indicates the friction force of pure gelatin gel (fat content = 0%). Other lines are shown to guide eyes.

For the bound droplet case (cf. Figure II - 8A) the decrease in friction force with increasing speed indicates that the friction behavior of gels with bound droplets and gelatin gels without fat are in the mixed lubrication regime of the Stribeck curve (Stribeck & Schröter, 1903). Since this part is in the mixed lubrication regime, both surface characteristics and lubricant viscosity are important (Dresselhuis *et al.*, 2007). The apparent viscosity of sheared gelatin gel mainly come from the gelatin matrix, and is independent of oil fraction (Chojnicka *et al.*, 2009). Therefore, the surface characteristics play a dominant role in the friction behavior of

the sheared gels. The surface characteristics may refer to those of the sample and those of the tribopair. We can exclude the latter because in our experiment we used PDMS probes with the same surfaces. Regarding the sample contributions we may distinguish presence of a lubricant, the characteristics of the lubricant and the interaction of the lubricant with the surfaces (Dresselhuis *et al.*, 2007).

For the unbound case (cf. Figure II - 8B) we find that the Stribeck curve is in the boundary regime. In the boundary regime only surface properties are important. Since we can exclude the tribopair influences, the surface properties are due to the presence of the lubricant, the characteristics of the lubricant and the interaction of the lubricant with the tribopair surfaces. Regarding the surface properties, the differences between bound and unbound lie in the ease of release of fat droplets and in the type of emulsifier used. It is possible that this easier release of fat droplets and the change of emulsifier (towards Tween 20) could change the surface properties of the contacting surfaces and create a boundary film easier. Such a boundary film would explain why the friction level in the boundary regime (unbound case) is lower than that in the mixed regime (bound case) as put forward by (Chojnicka-Paszun *et al.*, 2012). The first possible cause (ease of release of fat droplets) has been confirmed experimentally (see later in this section where we discuss the microstructural aspects as depicted in Figure II - 10 in more detail).

For both cases, bound and unbound, increasing fat content leads to a decreasing friction force. This effect is independent of the type of emulsifier. This suggests that with increasing fat content more fat is released from the gel matrix or more fat droplets coalesced, both enhancing the lubrication. We speculate that during shearing the gels with unbound droplets could release more fat. We expect that the friction force of gels with unbound droplets will be lower than with bound droplets. Indeed this is the case at a speed lower than 50 mm/s. At a higher speed, the friction force of gels with unbound droplets slightly increases. This may be attributed to the existing microstructure of the gel, leading to a release of gel particles that exhibit a larger friction.

Effects of amount of fat have also been described earlier. Dresselhuis found that in emulsions, when fat content is higher than 1% (up to 40%), increasing fat content does not decrease friction (Dresselhuis *et al.*, 2007). Chojnicka-Paszun found that the friction of milk was only independent of fat content up to a fat content of 1%. When fat content increased from 1% to 10%, the friction decreased (Chojnicka-Paszun *et al.*, 2012). In our study we saw that fat content has an impact on decreasing friction of emulsion-filled gels when fat content is above 5%. The difference between our results and the ones in the literature mentioned

might be due to a) the fact that our systems are gels while the ones in the literature refer to emulsions, and b) a different composition of the aqueous phase. Differences between gels and emulsions in parameters like viscosity of the system and mobility and aggregation of fat droplets are likely to play a role. In our study the system is a gel, and the final protein concentration (in aqueous phase, bound gels) is below 0.38%. Dresselhuis studied emulsions containing low amount of protein (0.3% and 1%), while Chojnicak-Paszun studied standardized milk containing 3.3% of protein. Chojnicak-Paszun suggested that the decrease in friction with increasing fat content is due to the increasing amount of fat that forms a boundary film and therefore provides better lubrication. This would explain our results as well.

Until now we have discussed the effects of unbound versus bound droplets, and the effects of amount of fat. The SFC in these systems have been all the same and relatively high (hard pork fat, 36%). We now discuss the effect of SFC in the droplets.

In Figure II - 9A (droplets of soft pork fat with 4% SFC), we find that for the bound droplet case, friction decreased with increasing moving speed from 10 to 80 mm/s, and with increasing fat content 5% to 20%. Comparing with Figure II - 8 we conclude that higher SFC yields a larger decrease of friction force versus speed. We also conclude that high SFC yields a larger decrease in friction upon increasing fat content than lower SFC. We also conclude that the friction force in the high SFC case is lower than the corresponding friction force in the low SFC case.

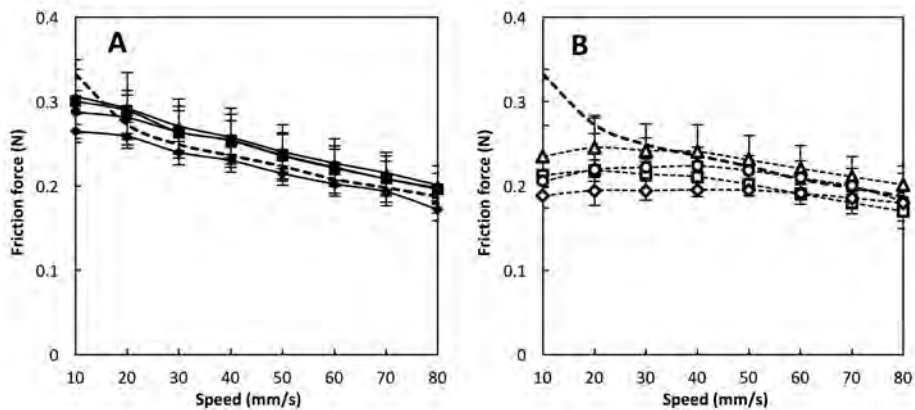


Figure II - 9 Friction force of pre-treated emulsion-filled gels containing soft pork fat (SFC=4%) as a function of speed. Filled symbols represent for gels with bound droplets (A), empty symbol represent for gels with unbound droplets (B). Fat content:  $\blacktriangle$   $\triangle$  =5%;  $\blacksquare$   $\square$  =10%;  $\bullet$   $\circ$  =15%;  $\blacklozenge$   $\diamond$  =20%. Discontinuous line without symbols indicates the friction force of pure gelatin gel (fat content = 0%). Other lines are shown to guide eyes.



In Figure II - 9B, we find that for the unbound droplet case, friction is not much influenced by speed, similar to the case of high SFC (see Figure II - 8B). We note that there are subtle dependencies of friction versus speed, which have been already discussed in the high SFC case and are similar to the low SFC. Comparing with Figure II - 8B we conclude high SFC yields a larger decrease in friction upon increasing fat content than lower SFC. We also conclude that the friction force in the high SFC case is lower than the corresponding friction force in the low SFC case.

In summary, we find that increase of SFC and fat content decreases friction. This is in line with our expectation B. In order to conclude on the validity of this expectation we need to validate expectation A, for which we need information on the micro-structural evolution.

The microstructure of these emulsion-filled gels containing hard pork fat (high SFC), mixed pork fat (medium SFC) and soft pork fat (low SFC) were observed before and after the tribological experiment in the OTC.

Before being put in the tribometer, the gel samples were pretreated by expelling them from a syringe, to mimic chewed foods under oral conditions. Figure II - 10 shows that fat droplets that were bound to the matrix were homogeneously distributed in the gel after pretreatment and before application of shear in OTC. We note that the homogeneous distribution also has been observed in the gels before pretreatment (results not shown). We conclude that pretreatment does not induce coalescence of bound droplets. In contrast, for fat droplets that were unbound and that had high and medium SFC, some droplet coalescence occurred after pretreatment. Fat droplets that were unbound with low SFC remained homogeneously distributed in the gel matrix. We conclude that during pretreatment the unbound droplets with high and medium SFC in filled gels were more prone to coalescence than bound droplets, and unbound droplets with low SFC.

From Figure II - 10 we conclude that bound droplets (with high and medium SFC) after application of shear in the tribometer show less coalescence than corresponding unbound droplets. This is probably caused by the fact that the bound droplets are protected against coalescence by a layer of gel material. There is no large difference for bound versus unbound for low SFC; in both cases there was negligible coalescence. This negligible coalescence at low SFC is due to low sensitivity to rupture of the droplet interface upon deformation because of the low presence of fat crystals inside the droplets.

Regarding the effect of SFC, we conclude from Figure II - 10 that high and medium SFC yields much more coalescence than low SFC (that shows negligible coalescence). This holds

for both bound and unbound. This effect of high and medium SFC is more pronounced in the unbound case, to the level of formation of streaks of fat. Low SFC shows negligible coalescence.

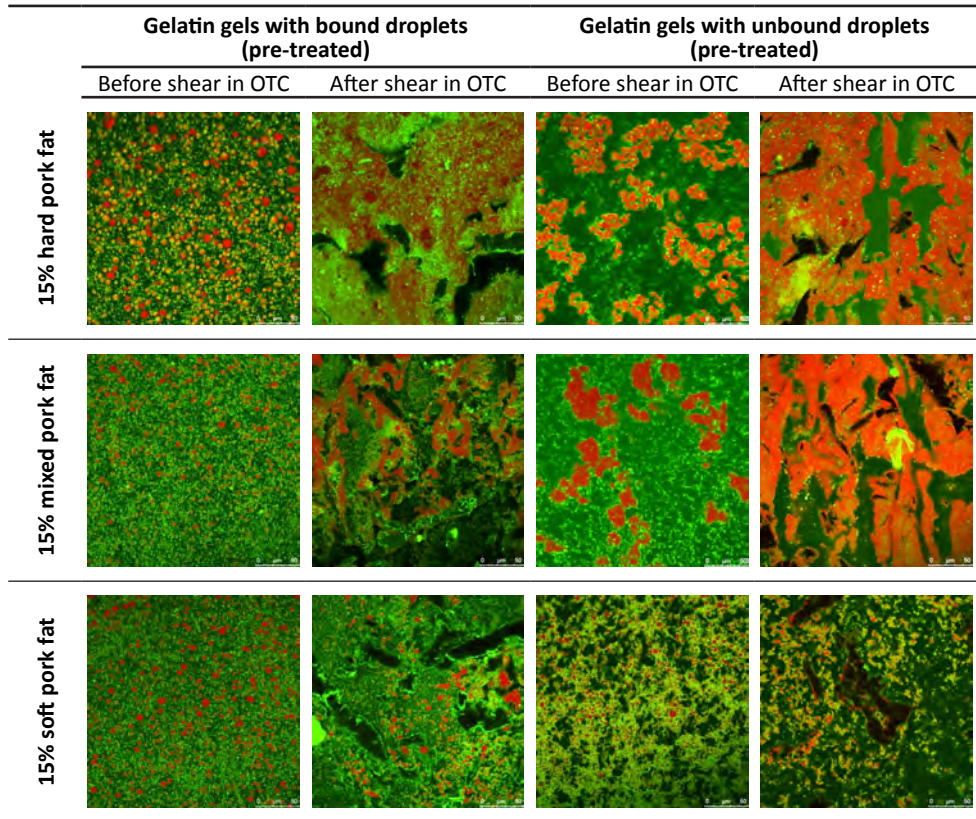


Figure II - 10 CLSM image of fat emulsion-filled gels (expelled from a syringe) before shear and after shear in a tribometer. Red phase represents fat, green is gel matrix. Image size is 250µm x 250µm.

From the results above, we conclude that unbound droplets and droplets with higher SFC yield lower stability against coalescence. These findings fulfill expectation A. As we mentioned above, in order to conclude on the expectation B we needed to validate expectation A (regarding structural information). Having now established the validation of expectation A we thus have concluded on the validity of expectation B.

In addition to the effects of SFC we briefly address the effects of fat content on microstructural evolution in relation to friction. We confine ourselves to the unbound case. Regarding the microstructural evolution, Figure II - 11 shows that higher fat content leads to more coalescence (for high and medium SFC). In relation to friction (cf. Figure II - 8 & Figure II - 9)

higher fat content leads to lower friction. We then conclude that more coalescence implies lower friction. This supports our conclusion on the validation of expectation B. For low SFC, no obvious coalescence is observed, regardless of fat content.

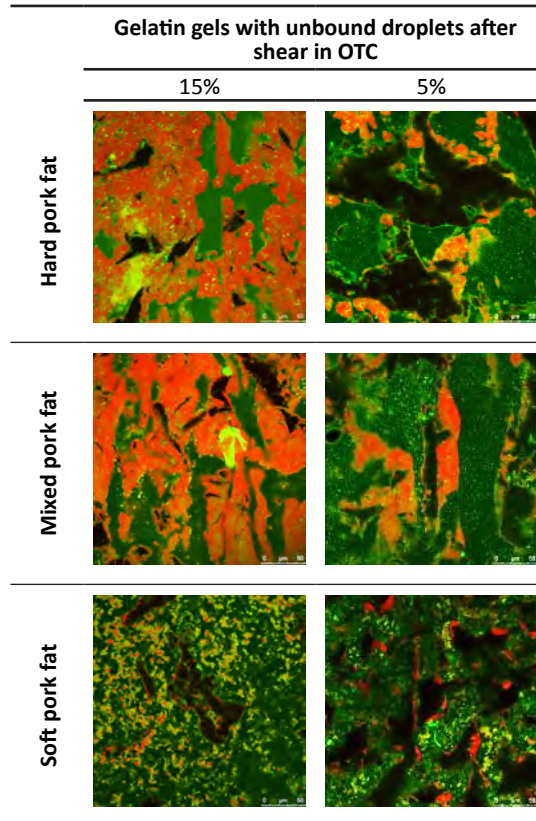


Figure II - 11 CLSM images of fat emulsion-filled gels (with unbound droplets, expelled from a syringe) after shear in a tribometer showing the effect of fat content of 15% and 5%. Red phase represents fat, green is gel matrix. Image size is 250 $\mu$ m x 250 $\mu$ m.

### 2.3.4 Sensory perception of emulsion-filled gels

During the QDA panel training sessions, 25 sensory attributes were generated (Table II - 2). Out of the 25 attributes, 14 attributes that are related to texture perception and fat sensations will be discussed (in the table they are marked with an asterisk). Mean sensory scores for emulsion-filled gels and ANOVA analysis are shown in Table II – 4 (same matrix concentration) and Table II – 5 (different matrix concentration but controlled gel modulus).

**Table II - 4 Mean sensory scores for emulsion-filled gels with same matrix (gelatin) concentration and ANOVA analysis. Significant differences indicated with asterisk: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Different small letters indicate a significant difference at  $p < 0.05$ .**

Attribute	A	B	C	D	E	F	G	H	I	X	Y	F-value	sign
<b>MF-firm</b>	59.77 b	60.33 b	53.85 bcd	43.45 cd	44.63 bcd	54.46 bc	24.67 e	26.25 e	38.29 de	59.85 b	80.34 a	21.58	***
<b>MF-elastic</b>	47.85 b	51.43 ab	49.17 b	40.63 bc	37.62 bcd	45.60 b	21.92 d	26.70 cd	26.65 cd	50.55 ab	66.65 a	13.39	***
<b>MF-brittle</b>	62.85 a	56.06 ab	51.65 abcd	47.47 abcd	51.46 abcd	48.54 abcd	36.74 bcd	33.11 cd	30.50 d	60.78 a	52.95 abc	5.44	***
<b>MF-pieces</b>	64.89 ab	58.80 bcd	62.40 abc	40.52 e	49.14 cde	48.18 de	21.82 f	22.01 f	19.67 f	62.00 abcd	74.60 a	39.24	***
<b>MF-spreadable</b>	30.55 de	34.76 cd	37.06 bcd	50.22 b	48.63 bc	45.51 bc	78.81 a	77.05 a	79.62 a	27.72 de	18.70 e	48.02	***
<b>MF-watery</b>	46.59 a	44.21 a	44.38 a	46.38 a	46.43 a	40.40 ab	29.53 bc	30.40 bc	21.20 c	48.65 a	41.08 ab	9.55	***
<b>MF-melting</b>	55.14 a	50.79 a	47.11 ab	58.19 a	55.96 a	48.34 ab	58.16 a	55.33 a	43.34 ab	52.15 a	35.44 b	4.58	***
<b>MF-fatty</b>	24.27 b	22.92 b	25.03 b	28.34 b	25.12 b	28.70 b	52.69 a	53.07 a	60.50 a	20.68 b	21.05 b	32.36	***
<b>MF-sticky</b>	24.84 bc	19.47 c	23.11 bc	24.13 bc	22.60 bc	30.20 abc	38.64 a	33.23 ab	39.32 a	21.97 c	20.21 c	8.56	***
<b>MF-slimy</b>	18.51 c	17.25 c	20.48 c	22.24 bc	21.34 bc	21.65 bc	32.05 a	24.80 abc	30.55 ab	18.30 c	20.30 c	5.29	***
<b>MF-slippery</b>	54.08 ab	52.57 ab	56.34 ab	44.65 bc	53.30 ab	47.04 ab	28.76 cd	27.49 d	28.49 cd	56.46 ab	62.15 a	11.45	***
<b>MF-creamy</b>	30.26 bc	31.79 bc	35.24 bc	41.95 b	37.62 b	40.02 b	68.08 a	61.56 a	67.22 a	23.07 c	21.63 c	30.75	***
<b>AF-fatty</b>	22.15 b	21.33 b	21.18 b	28.32 b	22.82 b	27.30 b	43.73 a	44.10 a	48.97 a	16.48 b	19.15 b	18.64	***
<b>AF-coating</b>	22.06 c	21.71 c	24.25 c	25.74 c	26.17 bc	27.72 bc	39.66 a	37.73 ab	47.31 a	24.67 c	22.44 c	11.36	***

**Table II - 5 Mean sensory scores for emulsion-filled gels with controlled modulus (different matrix concentration) and ANOVA analysis. Significant differences indicated with asterisk: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Different small letters indicate a significant difference at  $p < 0.05$ .**

Attribute	A	B	C	J	K	L	M	N	O	X	Y	F-value	Sign
<b>MF-firm</b>	59.38 bcd	54.24 cd	60.33 bcd	73.36 ab	76.7 a	75.35 a	58.1 cd	57.81 cd	67.57 abc	50.46 d	79.02 a	10.23	***
<b>MF-elastic</b>	48.15 bcd	44.79 cd	51.79 abc	61.93 ab	63.63 ab	67.48 a	34.96 d	39.7 cd	42.35 cd	45.27 cd	66.42 a	10.24	***
<b>MF-brittle</b>	62.46 a	57.2 a	55.05 a	49.49 ab	52.41 a	48.67 abc	32.87 bcd	32.11 cd	28.71 d	52.64 a	54.37 a	9.62	***
<b>MF-pieces</b>	63.3 a	60.67 a	62.48 a	61.22 a	63.27 a	68.59 a	27.55 b	29.88 b	28.8 b	62.57 a	74.3 a	25.66	***
<b>MF-spreadable</b>	33.15 b	35.15 b	33.14 b	31.85 bc	24.32 bc	25.85 bc	64.91 a	62.08 a	66.25 a	30.66 bc	17.49 c	26.57	***
<b>MF-watery</b>	50.4 ab	40.03 bcd	45.09 abc	30.9 def	33.39 cde	33.77 cde	20.81 efg	19.09 fg	17.14 g	57.25 a	43.87 abcd	20.32	***
<b>MF-melting</b>	56.22 a	55.5 ab	52.71 abc	41.62 bcd	41.88 bcd	33.92 d	38.24 d	36.91 d	31.76 d	59.51 a	39.64 cd	10.13	***
<b>MF-fatty</b>	23.44 cde	24.24 cde	20.9 de	39.51 b	30.97 bcd	33.78 bc	60.2 a	56.46 a	60.32 a	16.52 e	19.51 de	37.52	***
<b>MF-sticky</b>	23.94 bc	23.6 bc	20.36 bc	25.71 bc	27.76 b	27.4 bc	42.54 a	43.15 a	41.15 a	15.75 c	19.79 bc	13.96	***
<b>MF-slimy</b>	20.55 ab	18.65 b	18.34 b	23.3 ab	20.58 ab	19.74 ab	24.08 ab	28.95 a	25.25 ab	21.52 ab	22.37 ab	2.15	*
<b>MF-slippery</b>	59.96 a	47.99 a	55.59 a	50.98 a	54.64 a	59.05 a	28.72 b	29.31 b	22.23 b	57.74 a	61.35 a	14.77	***
<b>MF-creamy</b>	28.94 bcd	30.78 bcd	24.83 bcd	36.33 b	34.95 b	33.06 bc	61.08 a	55.32 a	61.51 a	17.78 d	19.3 cd	24.83	***
<b>AF-fatty</b>	20.91 cde	20.12 cde	21.09 cde	32.04 bcd	27.45 cde	32.68 bc	51.59 a	45.01 ab	49.03 a	18.5 e	18.93 de	19.28	***
<b>AF-coating</b>	25.55 c	23.44 c	24.79 c	31.73 abc	30.33 bc	32.82 abc	43.75 a	41.6 ab	41.03 ab	23.75 c	28.64 bc	6.88	***

Figure II - 12 shows the results of two principal component analysis (PCA) performed on the large deformation properties of the emulsion-filled gels and the scoring on sensory attributes. In Figure II - 12A all emulsion-filled gels have the same matrix concentration (4% gelatin) and different gel modulus (see Figure II - 2), while for Figure II - 12B the matrix concentrations of all emulsion-filled gels were adjusted to match for the gel modulus. These PCA plots show the relationships between sensory attributes and gel properties.

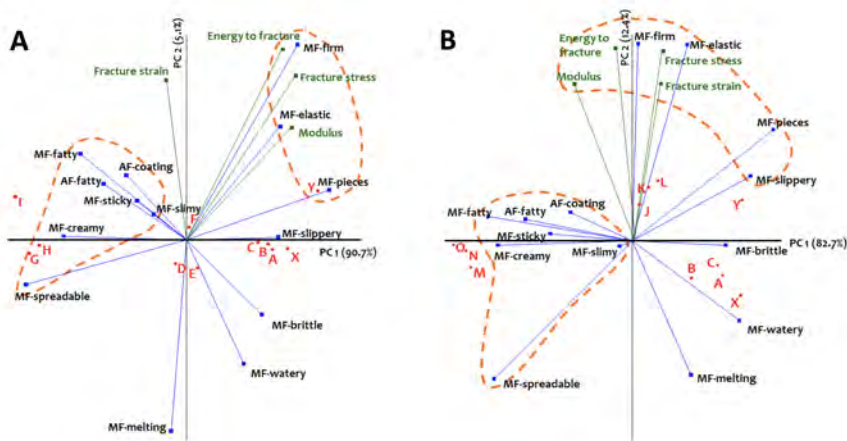


Figure II - 12 PCA Plots of samples with equal matrix concentration (4% gelatin) (A) and samples with controlled modulus (B). All sensory attributes are shown in black letters and blue dots, large deformation properties are shown in green, all samples are shown in red. For sample information please see Table II - 1.

Figure II - 12A shows a PCA plot that is based on all attributes generated from QDA but for the sake of clarity only the attributes related to fat perception are visualized. PC1 accounts for most of the variation (91%) between the gels, and distinguishes the gels varying from brittle/pieces/firm/elastic to fatty/creamy/spreadable. Fracture strain and mouthfeel MF-melting almost have no contribution to PC1, indicating that they have little impact on the perception of other attributes. On the right side of PC1, mouthfeel attributes MF-firm, MF-elastic, MF-pieces are positively related to each other and positively related to physical properties: the modulus, fracture stress and fracture energy. Most fat-related attributes are on the left side of PC1, including mouthfeel attributes MF-fatty, MF-creamy, MF-spreadable and after-feel attributes AF-fatty and AF-coating. Fat-related attributes are positively related to each other. The correlations were confirmed with the Pearson Linear Correlation analysis (Appendix Table II - B. 1 and II - B. 2), from which we can also see that fat-related attributes

are negatively related to the firm-related attributes that are opposite positioned on PC1.

In Figure II - 12B, we find that for samples with a controlled modulus, the loading plot containing all the attributes slightly rotates counterclockwise compared to samples with equal gel matrix concentration (Figure II - 12A). The contribution of fat-related attributes increases to the PC1 and the contribution of firm-related attributes increases to the PC2. In this plot, PC1 accounted for a cumulative variation of 83%. Compare to Figure II - 12A, the contributions of mouthfeel MF-firm, MF-elastic, and physical properties to PC1 are all decreased, meaning that the influence of physical properties on the fat-related attributes become less. We can see that the position of attributes, position of samples, and the correlation between them are in a similar pattern as in the Figure II - 12A. When the modulus is controlled (Figure II - 12B), the fat-related perceptions are increased at increasing fat content, and increased when the droplets are unbound (Table II - 1). We can already conclude that in our study the influence of fat droplets on the fat perceptions is not sensitive to changes in modulus or other texture properties.

In both Figure II - 12A and B, it can be seen that reference samples (X and Y), which are gelatin gels without fat, show the weakest fat-related perception intensities. Higher gelatin concentration (Y) gives rise to firmer and more elastic but less melting and creamy perceptions compare with 4% gelatin (X). All other samples contain 4% gelatin (Figure II - 12A) and different fat content. All gels that contain fat are distributed along PC1 into three clusters. Along the PC1 axis (from right to left, i.e. in the direction of increasing fat-related perceptions) these are: gels with 5% of bound fat droplets (A/B/C), gels with 5% unbound fat droplets (D/E/F), and gels with 15% unbound fat droplets.

Regarding the sensory perception, we conclude that a) gels with 5% unbound fat droplets (D/E/F) exhibit stronger fat-related perceptions (regardless of the SFC) than 5% bound fat droplets (A/B/C), b) gels with 15% unbound droplets (G/H/I) have even stronger fat-related sensory perception than those with 5% unbound droplets (D/E/F), and c) SFC does not influence fat-related perceptions. Regarding the microstructural evolution (from Figure II - 10) and friction (from Figure II - 8 & Figure II - 9) we already concluded for high SFC that d) gels with unbound droplets show more coalescence and a lower friction than bound droplets, e) gels with higher fat content show more coalescence and lower friction, and f) high SFC shows more coalescence and a lower friction.

According to expectation C, more coalescence and lower friction lead to a stronger fat-related perception. The combination of conclusions a) and d), as well as the combination of conclusions b) and e), fulfills our expectation C. However, the combination of conclusions

c) and f) does not fulfill our expectation C. We propose two possible reasons. One reason is that under in-mouth conditions solid fat crystals may melt, which reduces the instability against coalescence. Another (to our opinion more likely) reason is that expectation C holds for emulsions (Dresselhuis *et al.*, 2008c), while our systems are gels. The perception of fat and SFC in a gel is different from that in an emulsion because gels have a more complicated breakdown behavior than emulsions.

The effect of fat content is summarized in the combination of b) and e); higher fat content leads to stronger fat-related sensory perception, more coalescence and lower friction. We note that this is different from the results of Dresselhuis for emulsions. They did not find any effect of fat content on friction, but high fat content still resulted in stronger fat-related sensory perception. The absence of effect of fat content on friction again may be explained by the different breakdown behavior of gels versus emulsions. In gels, a release step of fat droplets has to occur, while for emulsions this is not the case. The fact that high fat content in emulsions still yields a stronger fat-related sensory perception could be explained by assuming that the intensity of the fat-related sensory perception increases with increasing time of a lubricating fat layer on the tongue under dynamic in-mouth circumstances.



## **2.4 Conclusions**

We have investigated in emulsion-filled gels where the droplets are bound to the gel matrix, and in emulsion-filled gels where the droplets are unbound. In the current work, we have chosen to vary the droplet instability by varying the SFC. We also varied the fat content.

We conclude that gels with unbound fat droplets exhibit stronger fat-related perceptions than bound fat droplets. This is in line with literature (Sala *et al.*, 2007b). In addition, we have found that this effect remains valid regardless of the SFC. We have been able to relate this increased fat-related sensory perception to increased coalescence and a lower friction.

We also conclude that gels (with unbound droplets) with higher fat content exhibit stronger fat-related sensory perception. This is in line with literature (Sala *et al.*, 2007b). We found in addition that this is also valid regardless of SFC. We have been able to relate this effect to more coalescence and lower friction. The identified relation between fat content, coalescence and friction that we found in our gel system is not reported for emulsions (Dresselhuis *et al.*, 2007). We conclude that this is due to the difference in breakdown behavior of gels and emulsions.

Thirdly, we conclude that SFC does not influence fat-related perceptions, in gels. We conclude however that gels with high SFC show more coalescence and a lower friction. Therefore, we are not able to establish a relation between the influence of SFC on fat-related perception with the influence of SFC on coalescence and friction, for our gel systems. In contrast, such a relation was reported in the literature, but for emulsions (Dresselhuis *et al.*, 2008c). The discrepancy between our findings and the literature is explained by the difference in breakdown behavior of gels and emulsions.

In addition, we find that fat-related sensory perception in our gel system is insensitive to changes in modulus or other texture properties (as modulated by fat content, interaction between droplet and matrix, and SFC).

## 2.5 Appendices

### Appendix II - A

Sample list and sample randomization during QDA profiling is shown in Table II - A. 1.

**Table II - A. 1 Sample list and sample code during the four sensory profiling sessions. Samples are alphabetically listed in this table (during the sensory profiling sessions samples were randomized except that the warm-up sample was presented firstly to the panelists).**

session 1		session 2		session 3 duplicate session 1		session 4 duplicate session 2	
Sample	code	Sample	code	Sample	code	Sample	code
A	875	J	684	A	542	J	102
B	971	K	265	B	637	K	830
C	319	L	803	C	841	L	798
D	495	M	257	D	954	M	897
E	713	N	804	E	704	N	567
F	138	O	850	F	925	O	139
G	631	A	521	G	792	A	963
H	185	B	162	H	134	B	238
I	240	C	481	I	730	C	723
X	591	X	370	X	273	X	540
Y	658	Y	692	Y	423	Y	247
Warm-up: D	493	Warm-up: D	397	Warm-up: D	650	Warm-up: D	783

Appendix II - B

Pearson linear correlations of sensory attributes and large deformation properties of emulsion-filled gels are shown in Table II - B. 1 and Table II - B. 2.

Table II - B. 1 Pearson linear correlation of sensory attributes and large deformation properties of emulsion-filled gels with same matrix concentration.  $|R| \geq 0.75$  are in light shade,  $|R| \geq 0.85$  are in darker shade.

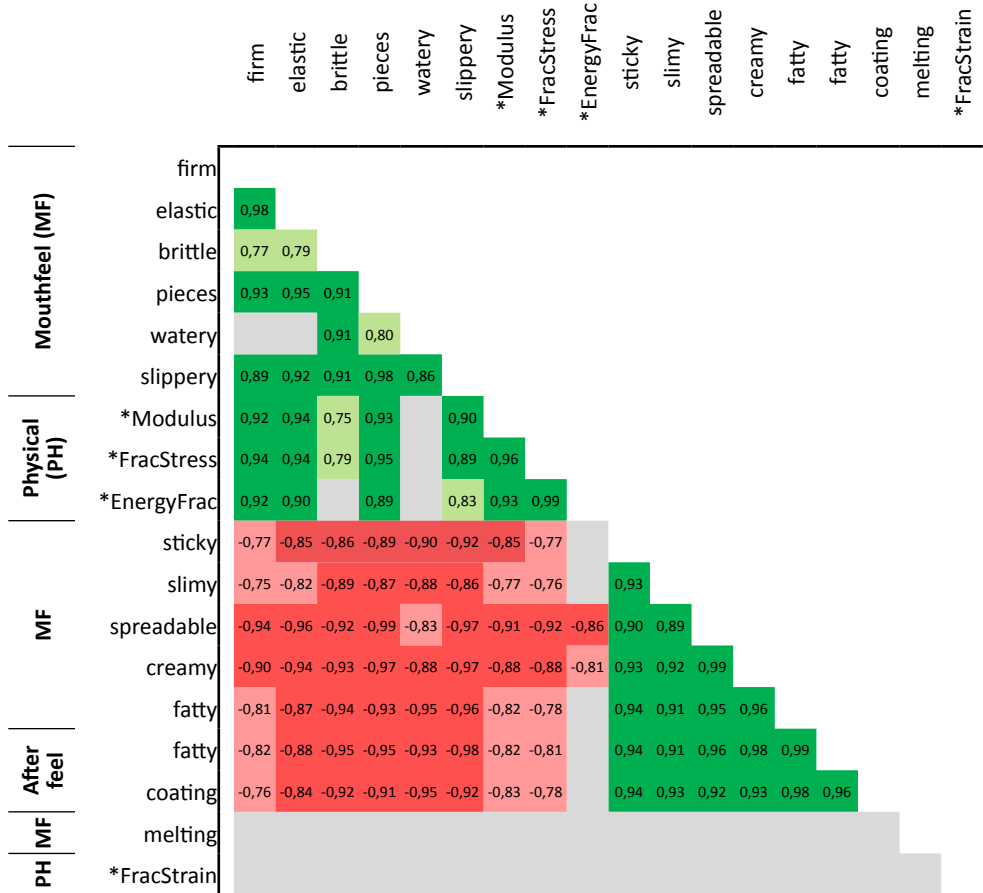
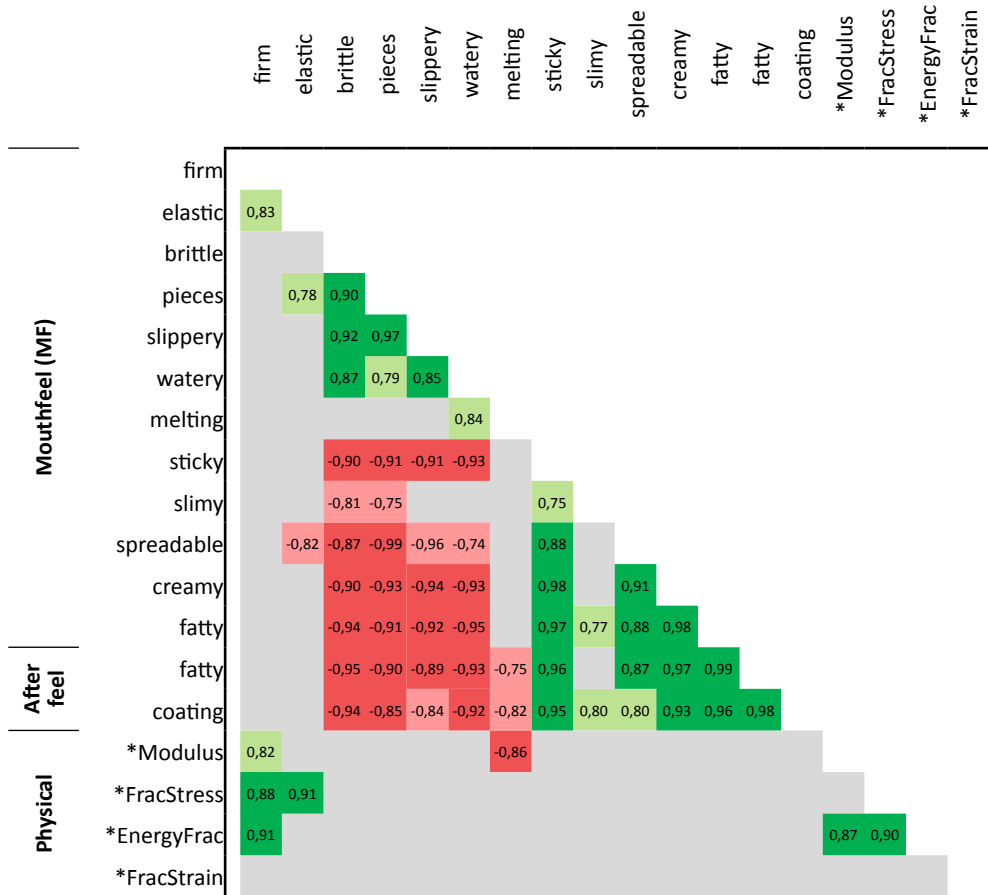


Table II - B. 2 Pearson linear correlation of sensory attributes and large deformation properties of emulsion-filled gels with adjusted matrix concentration (controlled modulus).  $|R| \geq 0.75$  are in light shade,  $|R| \geq 0.85$  are in darker shade.



Appendix II - C

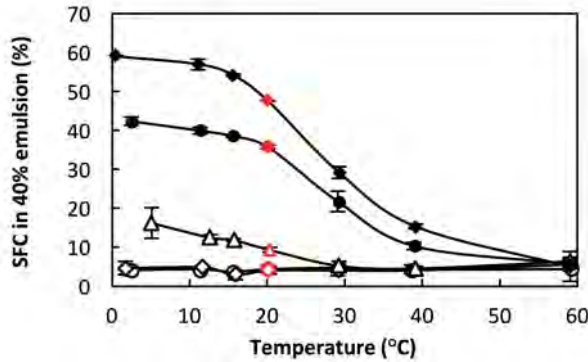


Figure II - C. 1 SFC of different types of fat droplets in 40% emulsion from 0°C to 60°C (◆ beef fat, ● pork fat, ▲ poultry fat, ◇ soft pork fat, ○ MCT oil).

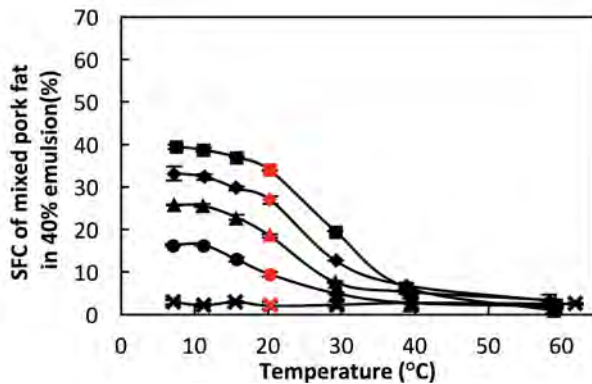


Figure II - C. 2 SFC of mixed pork fat droplets in 40% emulsion from 5°C to 60°C. The mixed pork fat was prepared by mixing hard pork fat and soft pork fat at different ratio (■ hard : soft = 100:0, ◆ 75:25, ▲ 50:50, ● 25:75, × 0:100).

The melting profile of different types of fats were determined with DSC. Method is described as follow: Equilibrate at -80.00°C → Isothermal for 10.00 min → Ramp 5.00°C/min to 80.00° → Ramp 5.00°C/min to -80.00°C → Equilibrate at -80.00°C → Isothermal for 10.00 min → Ramp 2.00°C/min to 80.00°C → Ramp 2.00°C/min to -80.00°C → Equilibrate at -80.00°C → Isothermal for 10.00 min → Ramp 10.00°C/min to 80.00°C → Ramp 10.00°C/min to -80.00°C). The results are shown in Figure C. 3 – Figure C. 6.

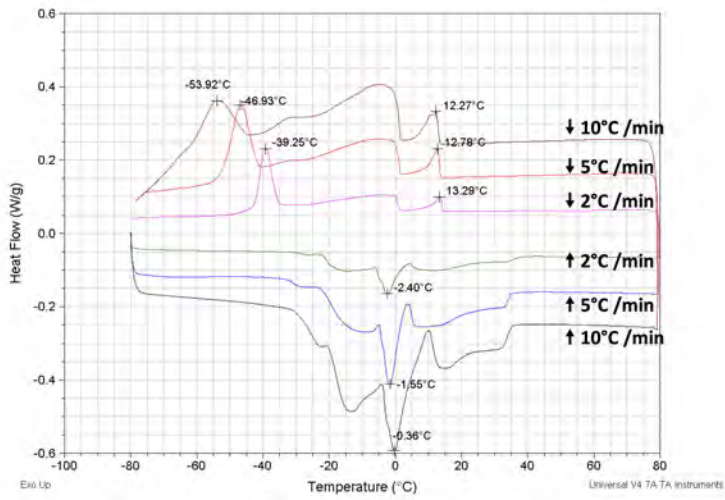


Figure II - C. 3 Melting profile of Poutry fat determined with DSC.

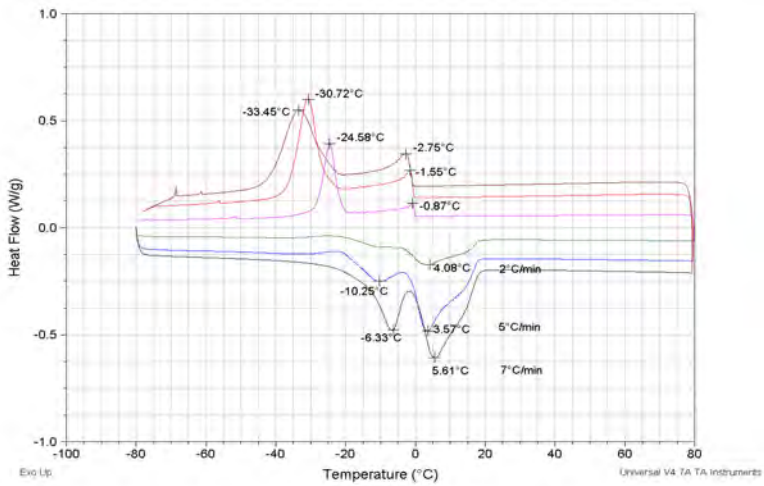


Figure II - C. 4 Melting profile of soft pork fat determined with DSC.

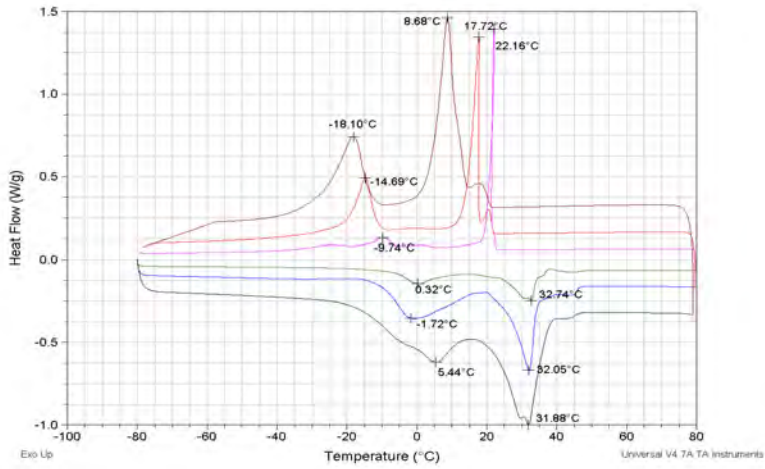


Figure II - C. 5 Melting profile of hard pork fat determined with DSC.

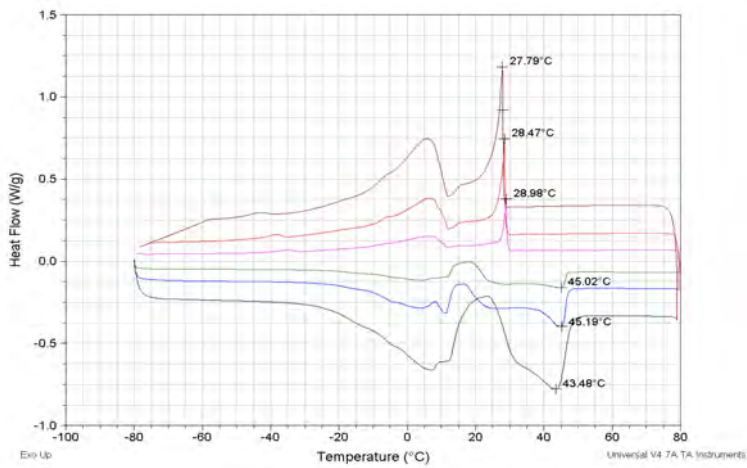


Figure II - C. 6 Melting profile of beef fat determined with DSC.





# Chapter 2 - Supplementary Results

## 2.7 Emulsion-filled whey protein gel

### 2.7.1 Introduction

In chapter 2, the effect of fat droplet characteristics on the large deformation properties of emulsion-filled gelatin gel was investigated. Gelatin gel is a polymer type of gel that is formed from a network of triple helices collagens. In this section, we investigated the effect of fat droplet characteristics on the large deformation properties of whey protein isolates gel, which is a particle type of gel. Gelation of whey protein isolates can be induced in several ways, such as heat-induced gelation and acid-induced cold gelation (Totosaus *et al.*, 2002). We used acid-induced cold gelation to avoid heating of emulsion droplets when making the emulsion-filled gels. For emulsion-filled gels, the emulsifier adsorbed at the surface of the droplets determines the interaction between fat droplets and gel matrix (Chen *et al.*, 1999). We attempted to make both bound and unbound type of droplets for whey protein gels. When using Tween 20 and sucrose esters as emulsifiers, it was possible to make the droplets unbound from the whey protein matrix, but the gel was not stable (creaming and phase separation). Thus, here we only discuss the results of emulsion-filled whey protein gels containing bound droplets.

### 2.7.2 Materials and methods

Whey protein isolate (WPI) was purchased from Davisco International Inc. (Bipro™, La Sueur, MN, USA). Glucono- $\delta$ -lactone (GDL) was obtained from Sigma Chemicals. Five types of fats were used for making o/w emulsions. These fats were the same as described in section 2.2.1. Solid fat content (SFC) of these fats in emulsions varied from 4.0 to 47.5% (Table II - 3).

To obtain emulsion-filled whey protein gel by cold gelation, the first step was to prepare whey protein aggregates (WPA). WPA were prepared according to the methods described by Alting *et al.* (2003) and Rosa *et al.* (2006). First, a 9% (w/w) whey protein isolate (WPI) solution was prepared by dissolving WPI in RO water and stirring for 2 hours at room temperature. This 9% (w/w) solution was then heated at 68.5 °C in a water bath for 2.5 hours and then cooled to 20°C. Next, the 9% (w/w) WPA solution was diluted with RO water to 4% (w/w). This 4% (w/w) WPA solution was used as both the gel matrix and emulsifier solution. A 40% (w/w) o/w emulsion was prepared by mixing molten fat or oil with this 4% WPA solution and homogenized at 300 bar (2<sup>nd</sup> stage 30 bar), yielding average droplet size of 1.5  $\mu\text{m}$ . The detailed homogenization step was the same as described previously (section 2.2.2). The obtained 40% (w/w) o/w emulsion was mixed with 4% (w/w) WPA

solution in different ratios to yield fat concentration of 0, 5, 10, 15, and 20% (w/w). The final concentration of WPA in the water phase, i.e. matrix concentration, was 4% (w/w). GDL powder was added to the mixed solution of fat emulsion and WPA in the appropriate amount to induce cold gelation. The final GDL concentration in the aqueous phase was 0.4% (w/w). This concentration of GDL yielded a final gel pH of 4.8 after gelation of WPA at 5°C for 20 h (in a refrigerator) and then at 20°C for 2 h (in a thermo-cabinet). Emulsion-filled WPA gels were gelled in 60 ml plastic syringes in a similar way as described for the preparation of emulsion-filled gelatin gels (section 2.2.3). Large deformation properties of emulsion-filled WPA gels were characterized using the same method as described in section 2.2.4.

### **2.7.3 Results and discussions**

Young's modulus of emulsion-filled WPA gels with varying SFC as a function of fat droplet concentration is shown in Figure II - 13A. Young's modulus increased with increasing fat droplet concentration for all types of fat, indicating that fat droplets are bound to the matrix. The fat droplets participated in building up the gel matrix through the emulsifier (WPA), which is the same as the matrix. This result agrees with the findings of (Sala *et al.*, 2007a). Young's modulus also increased with increasing SFC (filler modulus), in line with van der Poel's theory that predicts an increasing gel modulus with increasing filler modulus (van der Poel, 1958). This is also similar to our observation for the emulsion-filled gelatin gels (Figure II - 2A).

In Figure II - 13B, increasing the fat droplet concentration did not influence fracture strain of the WPA gel in the case of low SFC (< 10%), while it led to an increase in fracture strain in the case of high SFC (> 35%). This is different from what we observed for emulsion-filled gelatin gels, where fracture strain decreased with increasing fat droplet concentration (Figure II - 5). This is also different from the prediction of Nielsen theory, which describes the effect of bound filler particles to the polymer type of matrix on fracture strain (Nielsen, 1966). The gelatin gels studied previously is a polymer gel and it shows strain hardening, while the WPA gels studied here is a particle gel that shows strain weakening (stress-strain curves not shown). The difference in stress-strain relationship of the gelatin and whey protein matrices might be the reason for the discrepancy of the effect of filler concentration on their fracture strain. Increasing fat droplet concentration led to an increase of fracture stress, and this effect is more pronounced with increasing SFC (Figure II - 13C). This might be attributed to the fact that these bound droplets increased both the modulus (gel strength) and the fracture strain of the gels. Similar effect was observed for emulsion-filled WPA gel containing 9% (w/w) whey protein (Oliver *et al.*, 2015).

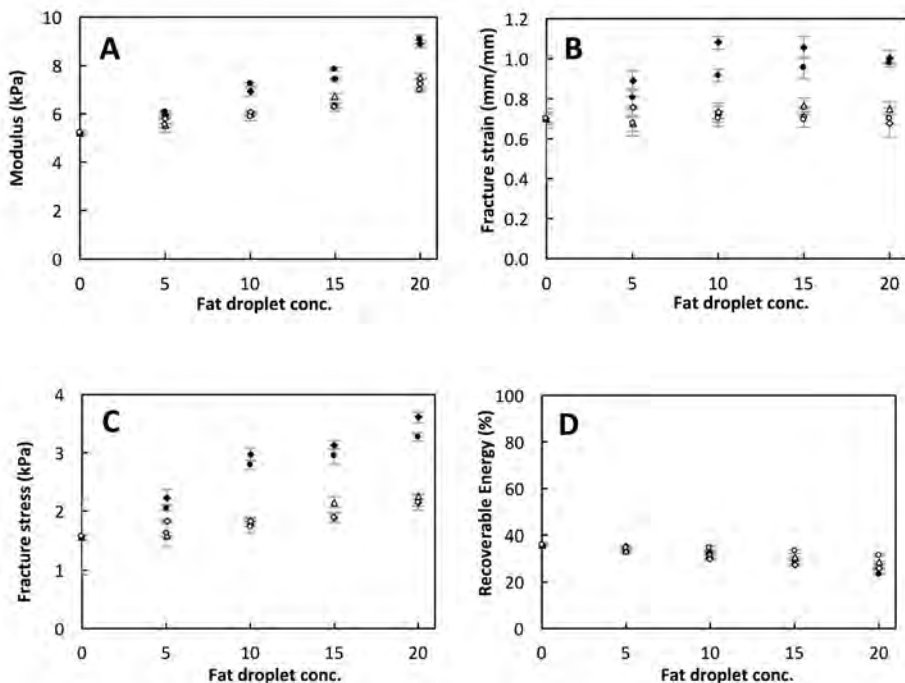


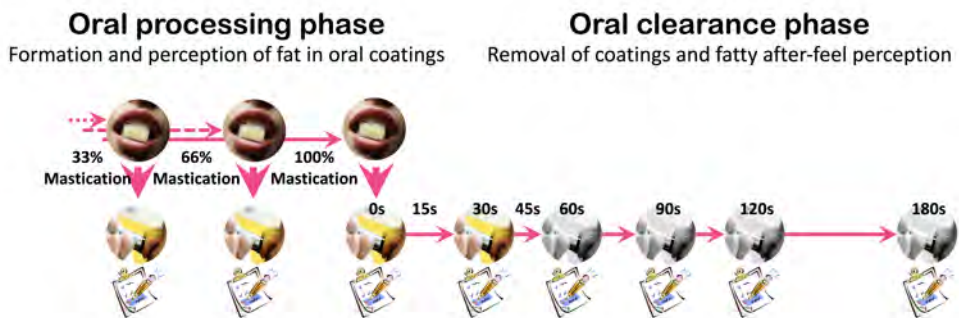
Figure II - 13 Large deformation properties of emulsion-filled whey protein aggregate gels as a function of increasing fat concentration (shown in w/w). A) Young's modulus; B) Fracture stress; C) Fracture strain; D) Recoverable energy. ◆ = beef fat, ● = hard pork fat, △ = poultry fat, ◇ = soft pork fat, ○ = MCT oil.

Figure II - 13D shows that neither increasing fat droplet concentration nor SFC had a considerable effect on the recoverable energy of WPA gels, confirming that the fat droplets are bound to the gel matrix. This is similar to the emulsion-filled gelatin gels. In general, the WPA gels had much lower recoverable energy (about 35%) than the gelatin gels (about 90%). This is because particle gels are less elastic and more viscous, which increases energy dissipation during the large deformation compression test (Guido Sala *et al.*, 2009a).

In conclusion, increasing bound fat droplet concentration and SFC increased Young's modulus, fracture strain and fracture stress of WPA gel, but did not influence the recoverable energy. Different from the strain-hardening of gelatin gels (polymer gels), emulsion-filled WPA gels (particle gels) showed a strain weakening behavior. This difference can probably explain the differences of filler concentration on the fracture strain between these two types of gels.

# Chapter 3

## Formation, clearance and mouthfeel perception of oral coatings formed by emulsion-filled gels



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**(\*The authors have contributed equally to this work)**

## Abstract

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

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

## Practical applications

Studies about oral coatings that are formed after consumption of semi-solids and solids foods are limited. This paper shows the possibility of characterizing the fat fraction in oral coatings after consumption of semi-solid food gels using *in vivo* fluorescence technique. Moreover, it provides knowledge on the dynamic formation and clearance of fat deposition in oral coatings of food gels. The importance of oral processing time as well as characteristics of fat droplets in food gels on the properties and perceptions of oral coatings is demonstrated. Together, this understanding on how fat behaves during oral processing creates valuable insights that guide the design of low-fat products with organoleptic properties of full-fat products.

### 3.1 Introduction

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

Previous studies have suggested that the release of fat from emulsion-filled gels and coalescence of fat droplets under mouth-mimicking *in vitro* conditions is related to a decrease in friction, leading to an increase in perception of fat-related sensory attributes (Dresselhuis *et al.*, 2007; Liu *et al.*, 2015; Sala *et al.*, 2007b). Evidence for an increase of the amount of fat deposited in the oral cavity during oral breakdown of emulsion-filled gels due to increased fat release and coalescence is not available. The oil fraction deposited on the tongue after consuming oil or o/w emulsions was quantified using an *in vivo* fluorescence methodology (Camacho *et al.*, 2014; Pivk *et al.*, 2008). They reported that oil fraction deposited on the tongue and fat-related mouthfeel and after-feel perception increased with increasing oil content in liquid o/w emulsions. Knowledge on the behavior and after-feel perception of oral coatings formed by liquids has increased in the last years. However, studies describing oral coatings formed by semi-solid and solid foods are scarce. To our knowledge, the study of Repoux *et al.* was the first and only one that investigated the formation of oral fat coatings formed by solid foods (cheeses) (Repoux *et al.*, 2012). In the mentioned study, the oral coatings were collected by asking the participants to rinse their mouth with water after masticating cheeses. The oral coatings were then quantified *ex vivo* in the rinsed water using fluorescence spectroscopy. Although this method is fast and easy to apply, it might have the

limitation that the rinsing of the coating is incomplete, so that some of the fat in the oral coating remains attached to the oral surface and is not quantified. Therefore, it is desirable to quantify oral coatings directly in mouth by *in vivo* fluorescence spectroscopy without an extraction/collection step.

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

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

We hypothesize that:

- 1a) Fat fraction deposited on the tongue and fatty mouthfeel perception increase during formation of oral coatings with increasing oral processing time;
- 1b) Fat fraction deposited on the tongue and fatty mouthfeel perception decrease during clearance of oral coating after expectoration of emulsion-filled gels;
- 2) Emulsion-filled gels with higher fat content and unbound fat droplets have higher fat fraction deposited on the tongue and higher intensity of fatty mouthfeel perception;



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

## 3.2 Materials and methods

### 3.2.1 Materials

Soft pork fat was kindly provided from Ten Kate Vetten B.V. (Ter Apelkanaal, The Netherlands). Porcine skin gelatin (bloom value 240-260) was kindly provided by Rousselot (Gent, Belgium). Powdered Whey Protein Isolate (WPI, Bipro™) was purchased from Davisco International Inc. (La Sueur, MN, USA). Tween 20 (Polyoxyethylene sorbitan monolaurate) and paraffin oil were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sweetener (Natrene, main components: cyclamate, saccharin and acesulfame-K), vanilla flavor (Dr. Oetker) were bought from local supermarkets (Wageningen, the Netherlands). Curcumin (7% Curcumin solution in propylene glycol and polysorbate, L-WS) was obtained from Sensient (the Netherlands). Pig's tongues were kindly donated by the VION Food Group (the Netherlands). All materials were used without further purification. All samples for sensory evaluation were prepared under food grade conditions. All samples were prepared with regular tap water.

### 3.2.2 Sample preparation

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

The emulsions were then mixed with gelatin solutions. Sweetener and vanilla flavor were added to the gel solutions to enhance the palatability of the emulsion-filled gels, and to mask the bitter taste of Tween 20 and the pork flavor of pork fat. The concentrations of

sweetener and vanilla flavor were determined by achieving similar taste and flavor intensity of the gels. The gel mixtures were immediately stored in the refrigerator at 5°C for 20 h and then kept at 20°C for at least 2 h prior to the sensory test and characterization.

**Table III - 1 Composition of emulsion-filled gels**

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

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

### 3.2.3 Characterization of emulsion-filled gels

Large deformation properties and microstructural analysis were carried out as described by Liu *et al.* (2015). An Instron universal testing machine (M5543, Instron International Ltd., Belgium) equipped with plate–plate geometry was used to perform uni-axial compression tests on emulsion-filled gels. The cylindrical gel specimen was 25 mm high, and the diameter of gel specimen was 26.4 mm. To determine Young’s modulus, fracture stress and fracture strain, all measurements were performed at a constant compression speed of 1 mm/s up to a compression strain of 80%. To determine recoverable energy, the measurements were performed at a constant compression and decompression speed of 1 mm/s up to a compression strain of 20%. Microstructural analysis of emulsion-filled gels were stained with 0.5% (w/w) Nile blue solution to visualize the fat phase. CLSM images were recorded on a LEICA TCS SP5 Confocal Laser Scanning microscope (Leica Microsystems CMS GmbH, Mannheim, Germany) equipped with an inverted microscope (Leica DM IRBE). The Argon laser and HeNe633 laser were used.

Friction force of the emulsion-filled gels was measured with a tribometer based on the method by Liu *et al.* (2015). The friction force of the small piece of intact gel was the same as the pre-treated gel (data not shown). For the convenience of measurement, instead of a

pre-treated gel, an intact piece of gel (about 200 mg) was sheared in the tribometer between a tribo-pair consisting of a glass plate and a PDMS probe. The load was set to 0.5 N. During each measurement the glass plate was oscillating at the speed 80 mm/s (Dresselhuis *et al.*, 2007). Friction force was determined during the shear movement. For each measurement a new probe was used and the glass surface was cleaned with water and ethanol. All the measurements were conducted at 20°C.

### 3.2.4 Quantification of *in vitro* fat release

To quantify the *in vitro* fat release from semi-solid emulsion-filled gels after shearing, the method developed by (Sala *et al.*, 2007b) was used. Emulsion-filled gels were squeezed out through the small orifice ( $d = 0.9$  mm) of a syringe by applying on the plunger a constant velocity (10 mm/s) with a Texture Analyzer (TA, Stable Micro Systems). A known amount of sheared gel (typically 15 g) was collected in a centrifuge tube and subsequently diluted by typically 30 g of demi water (to reach a 1:2 dilution ratio). The diluted sheared gel was gently vortexed for 15 s and then centrifuged at 3000 g for 5 min. The supernatant were filtered using Acrodisc syringe filters (5  $\mu\text{m}$  pore size, PALL Corporation) (Devezeaux de Lavergne *et al.*, 2015). The filters should allow the fat droplets that were released into the water phase to pass through the pore, since the fat droplet size was smaller than pore size. The fat content of the filtrate was quantified with the Röse-Gottlieb method (ISO 1211) by Qlip (Zutphen, The Netherlands). The fat release measurements per gel variant were conducted in triplicate. The quantification of fat content per filtrate was performed in duplicate. The measurements of fat droplet release were conducted at 20 and 37 °C.

### 3.2.5 *In vivo* determination of fat fraction deposited on the tongue surface

#### 3.2.5.1 *In vivo* fluorescence measurements

The method used in this study was previously described in detail (Camacho *et al.*, 2014). Fluorescence measurements were made with a single point measurement (Fluorolog Instruments SA Inc, Jobin Yvon Spex) at an excitation wavelength of 440 nm, an emission wavelength of 495 nm with a slit width of 0.95 mm and a measurement time of 0.1 s. To measure the fluorescence intensity on the tongue surfaces, fluorescence remote read fiber optic probe was used. To ensure the the distance between the probe and the surface remain constant, a plastic ring (diameter of 16.9 mm and height of 5 mm) was attached to the end of the probe. The probe was put gently on the front part of the anterior tongue during the measurements. To convert the fluorescence intensity measured on the subjects tongue

surface to the fat fraction deposited on the tongue surface, *i.e.* mass of fat per area of tongue ( $\text{mg}/\text{cm}^2$ ), calibration lines were made following the procedure previously described (Camacho *et al.*, 2014). In short, calibrations were made with pieces of the middle part of the pig's tongue at  $37.5^\circ\text{C}$ . The emulsion-filled gels were heated in a water bath, until they reached  $37^\circ\text{C}$  and were completely melted. The emulsion-filled gels were then kept at room temperature and spread on the surface of the pig's tongue ( $2\text{ cm} \times 2\text{ cm}$ ). The fluorescence intensity of the pig's tongue with the melted gel was measured. A fresh piece of tongue was used for each measurement. As the ingredients of each gel could affect the fluorescence intensity of curcumin, a calibration curve (fluorescent intensities vs. fat fraction on tongue surface) was made for each of the four gels. All calibration measurements were performed in triplicate, and calibration lines are shown in the appendices (Figure III - A1-A3).

#### 3.2.5.2 Selection of the subjects

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

Mastication behavior can vary between subjects and influence the fat fraction on the tongue and the perception of fat. In order to perform the measurements with a homogeneous panel, a pre-selection of subjects was conducted based on: (i) natural mastication time of the gel and, (ii) understanding of the attributes. The subjects were asked to measure the mastication time for two 5 mL emulsion-filled gels (gels with 15% bound droplets and gels with 15% unbound droplets – gels with the highest and lowest Young's modulus) until the natural intention to swallow the sample. Each emulsion-filled gel had a three-digit code and was presented to the subject in triplicate in random order. Subjects with a mastication time with a  $|z\text{-value}| \geq 2$  were excluded from the study. The average mastication time for the gels of the selected group was approximately 8 s ( $7.7 \pm 0.7$  s).

The selected subjects were invited for an introduction session during which the definition of the sensory attribute used in the study “fatty” and the evaluation protocol were explained (Table III - 2).

**Table III - 2 Definition of the sensory attribute and evaluation protocol.**

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

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

### 3.2.5.3 Determination of fat fraction deposited on tongue surface

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

### 3.2.5.4 Determination of mouthfeel and after-feel perception of oral coatings

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

#### 3.2.5.5 Determination of fat clearance due to follow-up consumption

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

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

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

#### 3.2.6 Statistical data analysis

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

SPSS® Statistics version 19 was used for the statistical data analysis of the results obtained from the sensory and *in vivo* fluorescence measurements. Descriptive statistics were used to obtain the mean and standard error (SE). Outliers ( $z > 2$ ) were removed from the data. Fluorescence intensity data of both formation and clearance of the coating and data for mechanical clearance was normalized with a square root transformation. The effect of gel (within subject factor; 5% unbound, 15% unbound, 5% bound and 15% unbound), time (within subject factor; 0, 15, 30, 45, 60, 90, 120, 180 s after expectoration or within subject factor: 2, 5 and 8 s mastication time) and interactions on fat fraction deposited on the tongue

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

### **3.3 Results**

#### **3.3.1 Characteristics of emulsion-filled gels**

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

##### **3.3.1.1 Large deformation properties**

Figure III - 1A shows that with increasing fat content from 5 to 15%, the Young's modulus of the emulsion-filled gels increases when fat droplets are stabilized with WPI, while decreases when fat droplets are stabilized with Tween 20. Young's moduli of gels with droplets stabilized with Tween 20 are lower than those stabilized with WPI. Figure III - 1B shows that for both gels with bound droplets and unbound droplets, fracture stress decreases significantly ( $p < 0.05$ ) with increasing fat content. The fracture stress of gels with bound droplets is larger than those with unbound droplets. Figure III - 1C shows a slight but significant decrease in fracture strain of gels with bound droplets as fat content increases ( $p < 0.01$ ), while the fracture strain of gels with unbound droplets is not significantly influenced by varying fat content from 5% to 15%. Figure III - 1D shows that the recoverable energy of emulsion-filled gels containing bound droplets is not significantly decreased with increasing fat content, while a significant decrease is observed when droplets are unbound from the gel matrix ( $p < 0.001$ ).

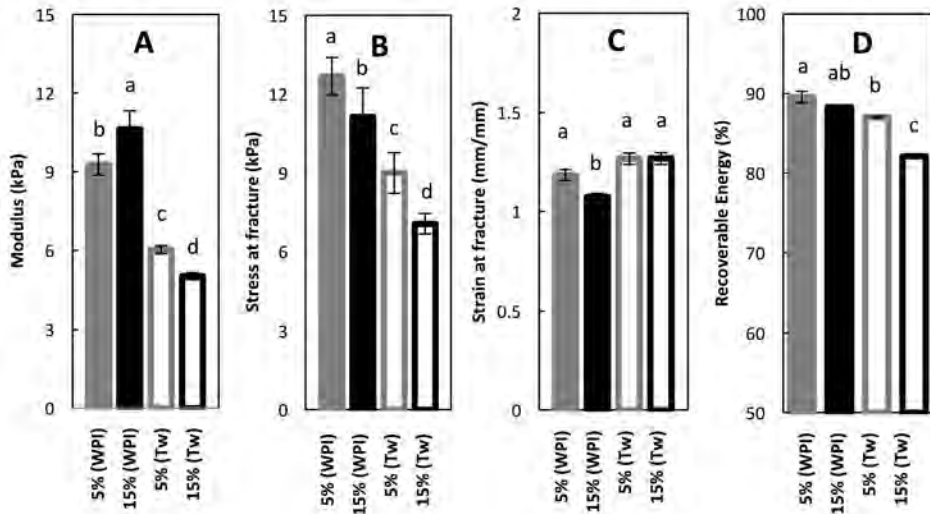


Figure III - 1 Large deformation properties of emulsion-filled gelatin gels. A) Young’s modulus; B) Stress at fracture; C) Strain at fracture; D) Recoverable energy. In each sub-figure: grey color = 5% fat; black color = 15% fat; filled bars = droplets stabilized with 1% WPI; empty bars = droplets stabilized with 2% Tween 20. Error bars represent standard deviation. Means that do not share letters are significantly different ( $p < 0.05$ ).

### 3.3.1.2 Microstructure

Figure III - 2 shows the microstructure of the four emulsion-filled gels. Droplets that are stabilized with 1% WPI (bound) are more homogenous distributed in the gel matrices than droplets that are stabilized with Tween 20 (unbound). Droplet aggregation is observed to a limited extent, but no coalescence of droplets is observed.

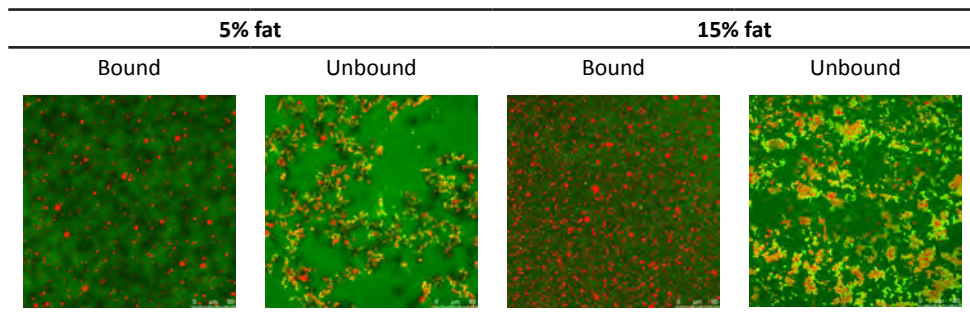
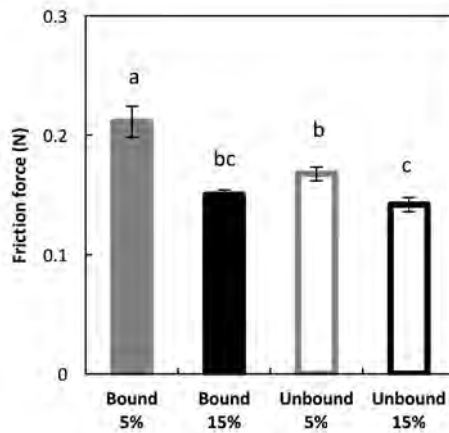


Figure III - 2 CLSM images of fat emulsion-filled gels. Red phase represents fat, green phase represent gelatin matrices. Image size is 160  $\mu\text{m}$  x 160  $\mu\text{m}$ .



### 3.3.1.3 Tribological properties

Figure III - 3 shows the friction force measured for an intact piece of gel sheared between two tribo-surfaces at 80 mm/s. For gels with bound droplets, increasing fat content from 5 to 15% leads to a significant decrease in friction force ( $p < 0.001$ ). For gels with unbound droplets, increasing fat content also leads to a significant decrease in friction force ( $p < 0.05$ ), but to a lesser extent than for gels with bound droplets. For gels with lower fat content (5%), the gel with unbound droplets has significantly lower friction force than the gel with bound droplets ( $p < 0.001$ ). For gels with higher fat content (15%), the gel with unbound droplets has a friction force that is not significantly different from the gel with bound droplets.



**Figure III - 3** Friction force of emulsion-filled gels measured at shearing speed of 80 mm/s. Error bars represent standard deviation. Means that do not share letters are significantly different ( $p < 0.05$ ).

### 3.3.1.4 *In vitro* fat droplet release

The fat droplets were extracted from emulsion-filled gels at 20 and 37°C. Figure III - 4 shows that at 20 °C, less than 0.4% fat was released from the sheared gels with 5% bound droplets, and less than 1% fat was released from the sheared gels with 15% bound droplets. For gels with 15% unbound droplets, significantly more amounts of fat were released from the gel matrices than gels with 15% bound droplets ( $p < 0.001$ ). The amount of fat released is proportional to the original fat content of the emulsion-filled gel. At 37°C, the fat droplet release for both unbound and bound droplets is the same and is proportional to the fat content of the emulsion-filled gel.

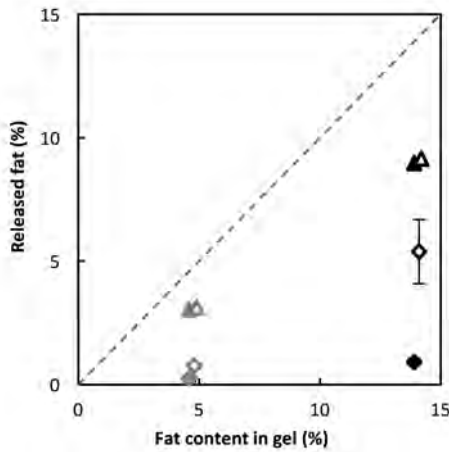


Figure III - 4 *In vitro* fat droplet release as a function of fat content in emulsion-filled gels. Diamond symbols represent 20 °C; triangle symbols represent 37 °C. Filled symbols represent bound droplets; empty symbols represent unbound droplets (slightly shifted to right to avoid overlapping with other points). Error bars represent standard deviation. Dashed line indicates total oil droplet release.

### 3.3.2 Formation of fat deposition on tongue during oral processing and mouthfeel perception

Figure III - 5A depicts the effect of oral processing time of the emulsion-filled gels on the formation of fat deposits on the back part of the anterior tongue. A significant main effect of sample [ $F(2.5, 96.5)=160.1, p < 0.001$ ] and time [ $F(2, 76)=3.2, p < 0.05$ ] on fat fraction was observed. No interaction effect of sample x time ( $p = 0.653$ ) was found on fat fraction.

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

Figure III - 5B shows the effect of oral processing time of the gels on the mouthfeel perception of the attribute fatty. Sample [ $F(3, 114)=27.0, p < 0.001$ ] and time [ $F(2, 76)=7.9, p = 0.001$ ] had a significant main effect on the perception of fatty mouthfeel of the coating. No interaction effect of sample x time ( $p = 0.121$ ) on the perception of fatty mouthfeel of the coating was found. The trend observed for the perception of fatty mouthfeel is similar to

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

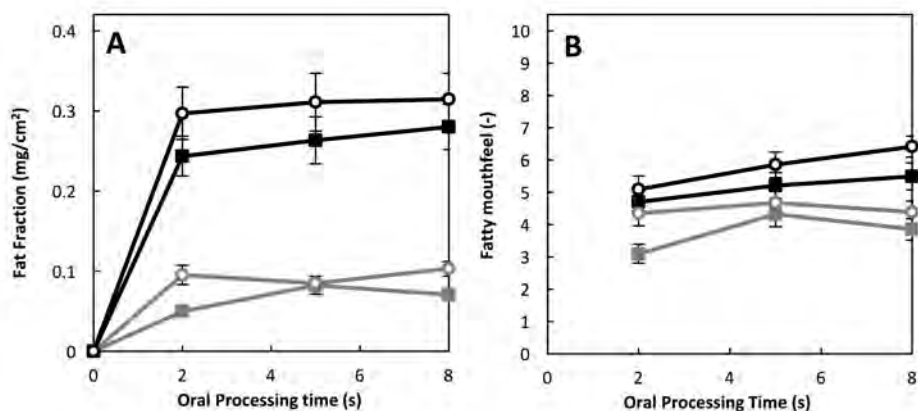


Figure III - 5 A) Fat fraction deposited on the back of the anterior part of the tongue as a function of oral processing time (s) of emulsion-filled gels. B) Fatty mouthfeel perception as a function of oral processing time (s) of emulsion-filled gels. Each data point represents the average of  $n = 13$  subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent standard error. (○ unbound 5%, ■ bound 5%, ○ unbound 15%, ■ bound 15%).

### 3.3.3 Clearance of fat deposition on tongue and after-feel perception

Figure III - 6A shows the clearance of fat fraction deposited on the tongue as function of time after expectoration of the gels. For these measurements, each gel was processed in mouth for 8 s (100% natural oral processing time) and then expectorated. Afterwards, the clearance of the fat coating was analyzed at different time points. A significant main effect of sample [ $F(3, 114)=52.7, p < 0.001$ ], time [ $F(2.6, 99.1)=204.8, p < 0.001$ ] and sample x time [ $F(7.4, 281.2)=20.2, p < 0.001$ ], on fat fraction was observed. Until 30 s after expectoration,

the fat fraction of gels with 15% fat remained significantly higher compared to gels with 5% fat ( $p < 0.05$ ).

Figure III - 6B shows the fatty after-feel perception as a function of time after the gel was expectorated. Results show a significant main effect of sample [ $F(3, 114)=18.3, p < 0.001$ ], time [ $F(1.7, 64.3)=243.2, p < 0.001$ ] and sample x time [ $F(7.6, 287.8)=6.1, p < 0.001$ ] on the fatty after-feel perception. Following the same trend as in the fat fraction clearance, the coatings formed by gels with 15% fat were perceived as significantly more fatty compared to the coatings formed by gels with 5% fat up to time 30 s after expectoration.

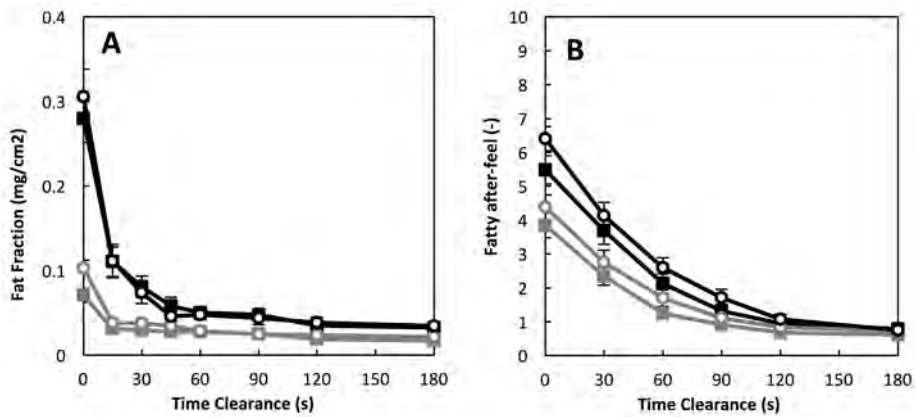


Figure III - 6 A) Fat fraction deposited on the back of the anterior part of the tongue as a function of time after each emulsion-filled gel was expectorated. B) Fatty after-feel perception as a function of time after each emulsion-filled gel was expectorated. Each data point represents the average of  $n=13$  subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent standard error. (○ unbound 5%, ■ bound 5%, □ unbound 15%, ● bound 15%).

### 3.3.4 Clearance of fat coating due to follow-up consumption

Figure III - 7 depicts the effect of different processing time of plain water or plain gelatin gel on the clearance dynamics of fat fraction deposited on the tongue. Significant main effect of water and gelatin [ $F(1, 38)=52.6, p < 0.001$ ], time [ $F(2.2, 82.2)=80.1, p < 0.001$ ] and water and gelatin x time [ $F(2.4, 91.7)=6.72, p = 0.001$ ], on fat fraction was observed. Clearance of fat fraction using water was more effective at every time point tested ( $p < 0.001$ ) compared to using gelatin gels. Longer oral processing time of both water and gelatin gel lead to a lower fat fraction (significantly decrease from 0 s till 2 s).

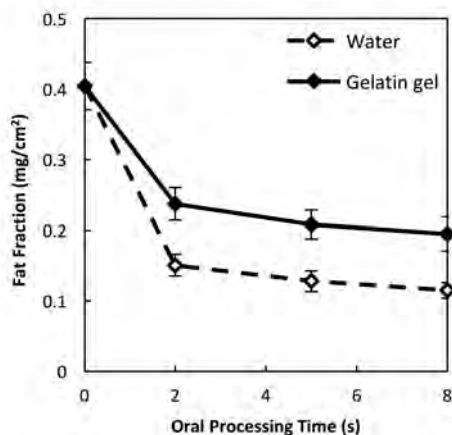


Figure III - 7 Fat fraction deposited on the back of the anterior part of the tongue as a function of processing time of water and gelatin gel. Time 0s corresponds to the fat fraction on the tongue after a 15% (w/w) fat emulsion stabilized with Tween 20 was processed in mouth for 30s and expectorated. Each data point represents the average of  $n=13$  subjects and 3 replicates. Lines are drawn to guide the eye. Error bars represent standard error.

### 3.4 Discussion

#### 3.4.1 Characteristics of emulsion-filled gels

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

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

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

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

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

### 3.4.2 Formation of fat deposition on the tongue and mouthfeel perception

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

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

be between 20 and 37°C. Therefore, the fat fraction on the tongue surface should correspond more accurately to the *in vitro* fat release at a temperature between 20 and 37°C.

The fatty mouthfeel perception followed the same trend as the fat fraction deposition on the tongue. In general, coatings with higher fat fractions led to higher intensities of fatty mouthfeel perception suggesting that the physical fraction of fat deposited on the tongue is sensed. Longer oral processing times led to more intense fatty mouthfeel perception. Likewise, fat droplets unbound from the matrix led to more intense fatty mouthfeel perception compared to fat droplets bound to the matrix. This is in accordance with previous research where a trained QDA panel evaluated comparable emulsion-filled gels (Liu *et al.*, 2015). This is also in accordance with the friction results (Figure III - 3). Gels that had lower friction were perceived more fatty.

To summarize, differences in intensities of fatty mouthfeel are mainly due to the differences in fat deposition on the tongue and the consequent differences in friction forces.

### **3.4.3 Clearance of fat deposition on the tongue and after-feel perception**

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

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

Fatty after-feel perception of the emulsion-filled gels followed the same trend as the fat fraction clearance. Up to 30 s after expectoration, the fatty after-feel perception of gels with 15% fat remained significantly higher compared to gels with 5% fat. Nevertheless, in contrast to the fat fraction clearance, there was no steep decrease on the fatty after-feel perception, but rather a smooth decrease. This is likely due to adaptation effects. Adaptation is a decrease in responsiveness under a constant stimulus (Lawless & Heymann, 2010). As the taste and mechanoreceptors have been continuously stimulated with the fat deposited on the tongue (throughout the mastication of emulsion-filled gels and after expectoration), it is possible that the subjects were less sensitive to be able to efficiently detect changes in the fat clearance from the tongue.

#### 3.4.4 Clearance of fat coating due to follow-up consumption

Figure III - 7 shows that water flow has a stronger effect on the removal of the fat deposited on the tongue than masticating a gelatin gel. Higher saliva flow was shown to lead to faster oral coatings' clearance compared to low saliva flow (Adams *et al.*, 2007). It is possible that the effect of water flow in-mouth is more effective compared to the effect of the mastication of the gel, which is likely to remove the fat deposited on the tongue due to abrasion by the pieces of gelatin during mastication. Nevertheless, at longer oral processing times, the gelatin gel melts in mouth and likely forms a melted gelatin layer. The gelatin layer might protect the fat deposited on the tongue from removal caused by, for instance, the mechanical rubbing of the tongue against the palate. Although water is more effective removing the fat deposited on the tongue, the fat is still not completely removed. Thus, studies which rely on the rinsing method to quantify coatings, *i.e.* removal of coating by rinsing with water, probably underestimate the fat content in the oral coating by 27.5 - 37.5 % due to incomplete removal.

Figure III - 7 shows a steep decrease of fat fraction deposited on the tongue after the gelatin gel and water were processed in mouth for 2 s (around 50% fat decrease). This suggests once more, that the first seconds after food consumption are the most relevant for the fat clearance either with or without the effect of follow-up consumption (Figure III - 6). Further, the oral processing time of water and gelatin gel did not create a significant effect on the fat coating removal after 2 s, indicating that the main cause of fat coating removal is the different in-mouth behavior of the water and gelatin gel.



### **3.5 Conclusions**

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

### 3.6 Appendices

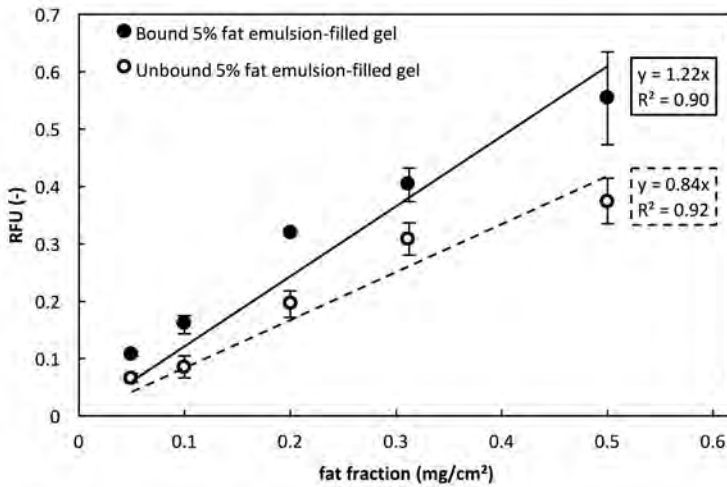


Figure III - A1 Calibration lines for 5% (w/w) fat emulsion-filled gels stabilized with Tween 20 (unbound) and with WPI (bound) – relating the relative fluorescence units (RFU) to the fat fraction on tongue surface (mg/cm<sup>2</sup>). Error bars represent the standard error.

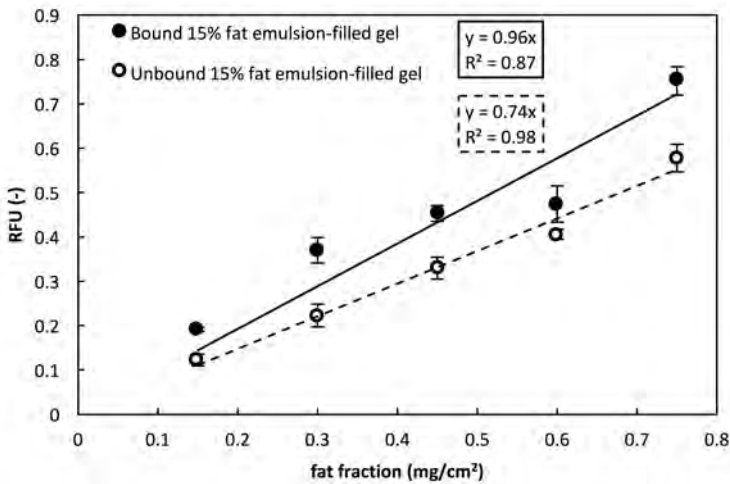


Figure III - A2 Calibration lines for 15% (w/w) fat emulsion-filled gels stabilized with Tween 20 (unbound) and with WPI (bound) – relating the relative fluorescence units (RFU) to the fat fraction on tongue surface (mg/cm<sup>2</sup>). Error bars represent the standard error.

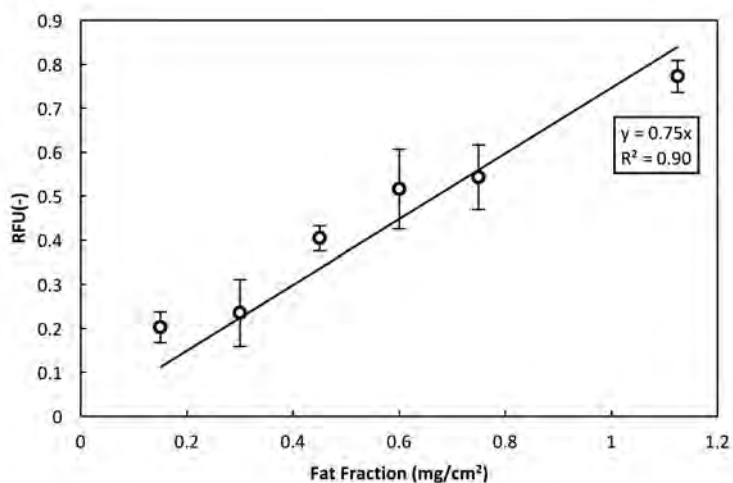


Figure III - A3 Calibration lines for 15% (w/w) fat emulsion stabilized with Tween 20 – relating the relative fluorescence units (RFU) to the fat fraction on tongue surface (mg/cm²). Error bars represent the standard error.

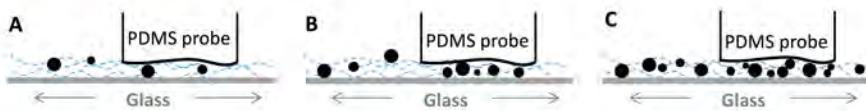


# Chapter 4

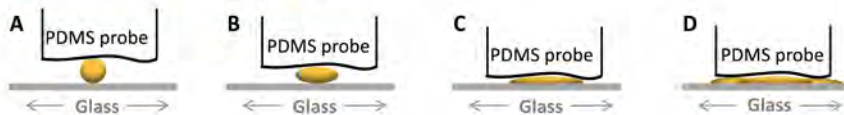
## Evidence for ball-bearing mechanism of microparticulated whey protein as fat replacer in liquid and semi-solid multi-component model foods

Mechanisms underlying lubrication behavior of multi-component food models

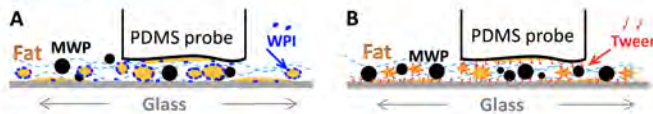
MWP: ball bearing



Fat droplet: plate out → film patches



Emulsifier layer



Published as:

Kun Liu, Yujie Tian, Markus Stieger, Erik van der Linden, Fred van de Velde, Evidence for ball-bearing mechanism of microparticulated whey protein as fat replacer in liquid and semi-solid multi-component model foods, *Food Hydrocolloids*, 2016, 52, 403-414.

## Abstract

The current understanding of the mechanisms underlying the fat mimicking properties of fat replacers, such as microparticulated whey protein (MWP), is limited. MWP is known to provide fat-related mouth-feel in specific foods, such as yoghurts and cheeses.

Tribological and rheological properties are well known to contribute to the perception of fat related sensory attributes. This study investigated the tribological and rheological properties of MWP in liquid and semi-solid model foods to reveal the mechanisms underlying the fat mimicking properties of MWP.

In liquids, addition of MWP reduced the friction coefficient effectively. After scaling out the impact of viscosity on lubrication, results provide strong evidence that the reduction of friction of MWP in liquids is mainly due to ball-bearing lubrication. In semi-solid gels, addition of MWP also decreased the friction, but to a smaller extent compared to liquid model foods. The mechanism underlying the lubrication behavior of multi-component semi-solid foods that contain fat droplets, emulsifier, fat replacers such as MWP embedded in a gel matrix is complex. We conclude that different components affect the lubrication properties of the composite food through different mechanisms. Fat droplets reduce friction due to the formation of a fat film following a plate-out mechanism. We suggest that emulsifiers influence the formation of a fat or emulsifier film, whereas MWP is suggested to reduce friction due to a ball-bearing mechanism. In addition, the influence of bulk viscosity, gel fracture behavior, and the interactions between the MWP and its surrounding matrix and emulsifiers, need to be accounted for.

## 4.1 Introduction

The consumer demand for healthier foods with lower amounts of fat is increasing (Malik *et al.*, 2013). Food industry continues to develop low-fat foods with reduced fat content while maintaining sensory properties. A simple reduction of fat content is often associated with undesirable changes in texture, sensory perception of fat-related sensory attributes and palatability of foods. In order to address this challenge, various ingredients with low or zero caloric value have been developed as fat replacers to substitute or mimic the properties of fats in foods. These fat replacers are based on lipids, carbohydrates or proteins (Chung *et al.*, 2013; Lucca & Tepper, 1994; Psimouli & Oreopoulou, 2013; Roller *et al.*, 1996). Although different fat replacers might lack similarities in physical and chemical structure with fats, under certain conditions they can replace specific functionalities of fats in foods. Fat contributes to many aspects in determining the overall properties of foods, such as appearance, flavor, texture, mouthfeel, and palatability of foods (Akhtar *et al.*, 2006; Chung *et al.*, 2013; McClements *et al.*, 1998). Among these properties, the impact of fat on the texture perception of foods, especially those relate to perception of mouthfeel attributes such as creaminess, is not fully understood.

Three categories of fat replacers have been suggested, based on different functionalities of fat in texture: thickening agents to control flow properties, bulking agents to enhance adsorption to the tongue, and microparticulated ingredients to improve lubrication properties (Roller *et al.*, 1996). This implies that fat replacement has to be approached in a comprehensive way. So far, no single fat replacer has been found mimicking the diverse functionalities of fat across a wide range of foods. The function of a fat replacer is apparently product and application specific. For example, starch is widely used as fat replacer in products for which the thickness perception are important. Starch may be less suitable for foods where mimicking of lubrication properties is more relevant.

Most fat replacers fall into the first two categories. Compared to the first two categories, the fat mimicking mechanisms of microparticulated fat replacers have not been investigated extensively. Despite the lack of this knowledge, the application of microparticulated fat replacers in several low fat food systems seems successful. Microparticulated fat replacers are mostly derived from proteins, such as whey protein, zein protein or egg white protein (Cheftel *et al.*, 1993). The reported microparticulates derived from zein protein and egg white protein, are both based on protein-polysaccharide complex formation (i.e. zein with carboxymethylcellulose, and egg-white protein with xanthan), while microparticulated whey protein (MWP) is only based on whey protein concentrate from milk. In this study, we

focus on MWP. It has been reported that MWP provides a smooth and creamy mouthfeel in low-fat foods (Cheftel *et al.*, 1993). MWP has been mostly applied in dairy based semi-solid and solid foods, including yoghurts (Tamime *et al.*, 1995; Torres *et al.*, 2009; Torres *et al.*, 2011), ice creams (N. S. Singer *et al.*, 1990) and cheeses (McMahon *et al.*; Sahan *et al.*, 2008; Singer, 1996; Sturaro *et al.*, 2015). MWP has also been applied in non-dairy products such as mayonnaise (Cheung *et al.*, 2002).

The perception of fat-related sensory attributes such as creamy provided by MWP has been hypothesized to be the consequence of ball-bearing lubrication caused by MWP (Cheftel *et al.*, 1993; Gaull, 1991). However, this proposed mechanism underlying the lubrication properties of MWP has not been verified experimentally yet. In addition, the tribological properties of MWP in different food matrices have not been studied. Studies about MWP as a fat replacer mostly focused on the effect of MWP on visual appearance (Chung *et al.*, 2014), viscosity (Cheung *et al.*, 2002; Chung *et al.*, 2014), microstructure and physical properties (Cheftel *et al.*, 1993; McMahon *et al.*, 1996; Schenkel *et al.*, 2013) as well as sensory properties (Cheung *et al.*, 2002; Torres *et al.*, 2011). The few studies investigating the tribological properties of related hydro-colloidal particles, such as kappa carrageenan based micro-particles (Garrec *et al.*, 2013), whey protein particles and aggregates (Chojnicka-Paszun *et al.*, 2014b; Chojnicka *et al.*, 2008), are restricted to liquid systems.

Under conditions relevant for oral processing of foods, the application of tribology in food research has revealed important correlations between lubrication properties and perception of fat-related sensory attributes in liquid emulsions (Chojnicka-Paszun *et al.*, 2012; Dresselhuis *et al.*, 2007; Kokini *et al.*, 1983) and semi-solid gels (de Wijk *et al.*, 2005; Kokini *et al.*, 1983; Liu *et al.*, 2015; Stokes *et al.*, 2013). In liquid emulsions, the sensitivity of oil droplets towards coalescence, which can be modified by changing droplet size, emulsifier type and concentration, solid fat content (SFC), was found to be correlated to a decrease of friction and an increase of fat-related perception (Chojnicka-Paszun *et al.*, 2012; Dresselhuis *et al.*, 2008c). In semi-solid emulsion-filled gels, as in a previous study that we have reported, the characteristics of fat droplets in the gel matrix could influence the tribological and sensorial properties of the overall gels (Liu *et al.*, 2015). For example, gels with unbound fat or oil droplets were perceived with higher intensity of fat-related sensory attributes regardless of the SFC, and these increased fat-related perceptions were correlated to lower frictions measured with a tribometer (Liu *et al.*, 2015). Therefore, to better understand the fat mimicking mechanisms of MWP in liquid and semi-solid foods, further knowledge is required on the tribological properties of MWP in different food matrices, and on the influence of matrix and oil droplet characteristics, such as viscosity,



fracture behavior, oil release and microstructure, on the lubrication properties.

The aim of this study is to better understand the tribological properties of MWP in liquid and semi-solid model foods. This knowledge should provide useful information to develop and select suitable fat replacers for specific food products with required texture and sensory properties. In this study a mouth-mimicking tribometer was employed to determine the tribological properties of MWP in four matrices: two liquid matrices (dispersions of MWP in water and MWP in o/w emulsions) and two semi-solid gel matrices (MWP in gelatin gels and MWP in emulsion-filled gelatin gels). For the liquid foods, viscosity is one of the key parameters that affect lubrication. Therefore, we also take into account the contribution of viscosity to lubrication. For the semi-solid foods, the mechanical breakdown behavior of the gels is studied because we hypothesize that that this is expected to have an impact on the lubrication properties.

## **4.2 Materials and methods**

### **4.2.1 Materials**

Pork fat (Sonac edible lard max 1) was supplied by Sonac (Son, the Netherlands). Porcine skin gelatin (bloom value 240 - 260) was supplied by Rousselot (Gent, Belgium). Powdered whey protein isolate (WPI, Bipro™) was obtained from Davisco International Inc. (La Sueur, MN, USA). Tween 20 (Polyoxyethylene sorbitan monolaurate) and paraffin oil were purchased from Sigma-Aldrich (St. Louis, MO, USA). Microparticulated whey protein (MWP) (Simplese® 100) was provided by CPKelco (Lille Skensved, Denmark). All materials were used without further purification. All samples were prepared with reverse osmosis water.

### **4.2.2 Sample preparations**

In Table IV - 1, the compositions of all samples are listed.

#### **4.2.2.1 Preparation of MWP dispersions**

MWP stock dispersion was prepared by dispersing 10% (w/w) MWP powder in water under continuous stirring for 3 h at room temperature. The stock dispersion was diluted with water to lower concentrations. All MWP dispersions were freshly prepared on the day of the measurement and were stirred until the moment of further analysis to avoid sedimentation.

Table IV - 1 Overview of the composition of all samples

MWP % w/w	Fat % w/w	Emulsifier (water phase, % w/w)	Gelatin (water phase, % w/w)	Fat droplet-gel matrix interaction
<b>MWP water dispersions</b>				
0.5; 1; 2; 4; 8	-	-	-	-
<b>MWP o/w emulsions</b>				
0; 1; 2; 4	10	1% WPI		
0; 1; 2; 4		2% Tween 20		
0; 1; 2; 4	20	1% WPI	-	-
0; 1; 2; 4		2% Tween 20		
0; 1; 2	36	1% WPI		
0; 1; 2		2% Tween 20		
<b>MWP in gelatin gels</b>				
0; 1; 2; 4; 6; 8	-	-	4	-
<b>MWP in emulsion-filled gels</b>				
0; 1; 2; 4; 6	5	1% WPI		Bound
0; 1; 2; 4; 6	15			
0; 1; 2; 4; 6	5	2% Tween 20	4	Unbound
0; 1; 2; 4; 6	15			

#### 4.2.2.2 Preparation of MWP o/w emulsions

A stock o/w emulsion of 40% (w/w) pork fat was prepared with one of two types of emulsifiers: either 1% (w/w) whey protein isolate (WPI) or 2% (w/w) Tween 20 was used in the aqueous phase. The 1% (w/w) WPI solution was made by dissolving and stirring the powdered WPI in water at room temperature for 1 h. The 2% (w/w) Tween 20 solution was prepared similarly. Pork fat was melted in a waterbath at 70 °C before homogenization. O/w emulsions (40% w/w) were prepared by mixing the molten fat with the emulsifier solutions. Emulsifier solutions were pre-heated before mixing with the fat. After pre-homogenization with an Ultra Turrax Polytron (Kinematica AG, Switzerland) for 2 min, a lab homogenizer Ariete (NS1001L-Panda, Niro Soavi, Parma, Italy) was used at homogenization pressures of 180 bar (Tween 20) and 310 bar (WPI) to produce the o/w emulsions with a comparable droplet size. The lab homogenizer was pre-heated by passing through hot water for 3 min before homogenization.

A 20% (w/w) MWP stock dispersion was prepared similarly as described above. All MWP-o/w emulsions were prepared by mixing the stock o/w emulsion, stock MWP dispersion and a stock emulsifier solution containing either 1% (w/w) WPI or 2% (w/w) Tween 20 at

calculated ratios. The ratio of mixing is calculated to yield same emulsifier concentrations in the aqueous phase for all o/w emulsions. All MWP-o/w emulsions were freshly prepared on the day of measurements and were kept under stirring until the moment of analysis to avoid sedimentation.

#### 4.2.2.3 Preparation of MWP gelatin gels

The previously described 10% (w/w) MWP stock dispersion was mixed with gelatin solutions at different ratios. The final concentration of gelatin in the aqueous phase was 4% (w/w). Gelatin was first dispersed in water and hydrated for 2 h at room temperature. Prior to mixing with MWP, gelatin was molten by heating at 80 °C for 30 min in a water bath and cooling to 35 °C by running tap water. After mixing with the MWP dispersion, the mixtures were stirred for 10 min to keep homogeneous and were further cooled to 28 °C. The mixtures were stored in the refrigerator at 4 °C to form gels. Gels were stored at 4 °C for 20 h and then kept in a thermo-cabinet at 20 °C for 2 h prior to further analysis.

#### 4.2.2.4 Preparation of MWP emulsion-filled gelatin gels

The previously mentioned 40% (w/w) stock o/w emulsions stabilized with WPI or Tween 20 were mixed with gelatin-MWP solutions at different ratios. The final concentration of gelatin in the aqueous phase of the gel was 4% (w/w). The gelatin-MWP solutions were prepared as described before. A 20% (w/w) MWP stock dispersion and a stock emulsifier solution were added into the gelatin solutions at calculated ratios. The ratio of mixing is calculated to make the emulsifier concentration in the final aqueous phase the same for all gel samples. The mixtures were stirred for 10 min and cooled to 25 °C before mixing with emulsions. The mixtures were stored in the refrigerator at 4 °C. Gels were stored at 4 °C for 20 h and then kept in a thermo-cabinet at 20 °C for 2 h prior to further analysis.

### 4.2.3 Sample characterization

#### 4.2.3.1 Characterization of fat droplet and particle size

The droplet size distribution of stock o/w emulsions and the particle size of MWP dispersions were measured using a MasterSizer2000 (Malvern Instruments Ltd., Malvern, UK). The 40% (w/w) stock o/w emulsions were first diluted 5 times by water before adding to the MasterSizer chamber. Samples were added into the chamber until an obscuration rate was reached between 10% and 12%. Volume-to-surface diameter ( $d_{3,2}$ ) was used as the parameter characterizing mean droplet size or particle size. A refractive index of 1.48,

1.47, and 1.33 was used for the fat droplet, MWP and the aqueous phase, respectively. For both fat droplet and MWP, an absorption of 0.01 was used. The calculation of fat droplet and MWP particle size distribution was based on a general purpose model distribution with fitting accuracy above 99%.

### 4.2.3.2 Characterization of microstructure of MWP emulsion filled gelatin gels using CLSM

Emulsion-filled gels that contain MWP were stained with 0.2% (w/w) Nile blue solution to visualize the fat and protein phase. CLSM images were recorded on a LEICA TCS SP5 Confocal Laser Scanning Microscope (Leica Microsystems CMS GmbH, Mannheim, Germany) equipped with an inverted microscope (Leica DM IRBE). An Argon-HeNe633 laser was used. The objective lens used was HCX PL APO 63×/1.20 Water CORR CS (Leica). Digital images were acquired at a resolution of 1024 × 1024 pixels.

### 4.2.3.3 Characterization of microstructure of MWP particles using SEM

Scanning Electron Microscopy (SEM) was used to determine the microstructure of the MWP particles (Phenom tabletop G2 pure Scanning Electron Microscope, Phenom-World BV, The Netherlands). MWP powder (without any pre-treatment) was directly placed on a carbon adhesive pad that was stuck to a stub (d = 12.7 mm). The stub was then placed in a standard sample holder (Phenom-World BV, The Netherlands). SEM image magnification was adjusted with a rotary knob.

### 4.2.3.4 Characterization of rheological properties of MWP dispersions and MWP o/w emulsions

The flow curves of MWP dispersions and MWP o/w emulsions were determined using a rheometer (Physica MCR 501, Anton Paar, Graz, Austria) at 20 °C. A concentric double gap cylinder geometry was used. Continuous flow measurements were performed. The shear rate was increased in logarithmic steps recording 10 data points per decade from 1 s<sup>-1</sup> to 1000 s<sup>-1</sup> in 10 min (upwards and downwards). Each measurement was repeated twice and the average values of viscosity as function of shear rate were reported.

### 4.2.3.5 Characterization of large deformation properties of MWP gelatin gels and MWP emulsion-filled gelatin gels

Uni-axial compression tests were performed on MWP gelatin gels and MWP emulsion-filled gelatin gels at 20 °C using an Instron universal testing machine (M5543, Instron International

Ltd., Belgium) equipped with plate–plate geometry. The diameter of the cylindrical gel was 26.4 mm and the height of the specimen was 25 mm. Both the top and bottom of the gel surfaces were lubricated with a thin layer of paraffin oil to prevent friction between sample and plate during compression. For the determination of Young's modulus, fracture stress and fracture strain, measurements were performed at a constant compression speed of 1 mm/s up to a compression strain of 80%. For the determination of recoverable energy, measurements were performed at a constant compression and decompression speed of 1 mm/s up to a compression strain of 25%. The ratio between the energy released during the decompression and the energy needed to compress the gels was defined as the recoverable energy. All measurements were conducted six to eight times and the average values were reported.

#### 4.2.3.6 Characterization of tribological properties of all samples

The tribological properties of all samples (MWP dispersions, MWP o/w emulsions, MWP gelatin gels, emulsion filled MWP gels) were determined with an optical tribological configuration (OTC) (Dresselhuis *et al.*, 2007; Liu *et al.*, 2015). A fixed amount of liquid (200  $\mu$ L) or an intact piece of gel (around 200 mg) was sheared in the OTC between two surfaces. In this study the gel piece was not pre-treated by pre-shearing prior to the tribological measurements. The lower surface is made of glass and the upper surface is a flat-bottom rough PDMS probe (Sylgard 184 Dow Corning, USA; mixing ratio of PDMS: cross linker = 10:1; diameter 6 mm). The load ( $F_z$ ) applied between the upper surface and the sample was 0.5 N. The glass surface was oscillated over a distance of 16 mm at an increasing oscillating speed from 10 mm/s to 80 mm/s with incremental steps of 10 mm/s. Each speed was kept for 10 oscillation cycles. The friction force ( $F_x$ ) was measured during the movement of the glass surface. Friction force at each speed was reported as the average value of 10 cycles. Each measurement was performed at 20 °C with a new PDMS probe. Prior to each measurement the glass surface was cleaned with ethanol and water. All measurements were conducted in triplicate.

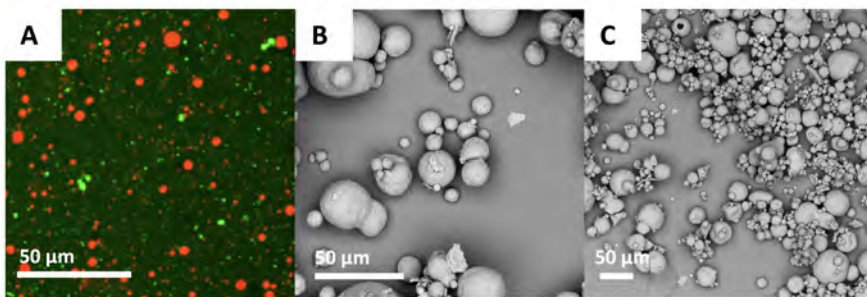
## 4.3 Results and discussions

### 4.3.1 Characterization of MWP particles and MWP emulsion-filled gelatin gels

The mean fat droplet size of the stock o/w emulsion was  $1.8 \pm 0.4 \mu\text{m}$ . MWP dispersions had a bi-modal particle size distribution based on volume frequency with two peaks at around

0.4  $\mu\text{m}$  and 7.0  $\mu\text{m}$ . The size distribution is in line with ranges mentioned in literature (0.1 - 3  $\mu\text{m}$ ) (Cheftel *et al.*, 1993; Gaull, 1991). Particle sizes of MWP of around 30  $\mu\text{m}$  were reported previously due to aggregation (Chung *et al.*, 2014). MWP particle size can be reduced by homogenization (Chung *et al.*, 2014). To protect the intact shape of MWP particles, no further homogenization was performed in the current study.

Figure IV - 1A shows the MWP particles embedded in an emulsion filled gelatin gel obtained by CSLM. The particle size of MWP is comparable to fat droplets prepared in this study. Both fat droplets and MWP particles were homogeneously distributed in the gelatin matrix. With increasing MWP content in the gel, more MWP particle aggregates were formed (micrographs not shown). Fat droplet aggregation and coalescence was observed in gels where fat droplets are not bound to the gelatin matrix (Tween 20 as emulsifier) which was due to the high solid fat content of the pork fat used (Liu *et al.*, 2015).



**Figure IV - 1** Microstructure of MWP. A) CLSM image of MWP emulsion-filled gelatin gel. Fat droplets are red, MWP particles are bright green. B) and C) SEM images of MWP particles (dry powder). Scale bar in all pictures represents 50  $\mu\text{m}$ .

SEM images of the MWP particles (Figure IV - 1B & C) show that the MWP particles are mostly spherical with smooth surfaces. Some aggregated particles can be observed, which are probably formed during the drying process during production of the MWP powder. The MWP particle size observed with SEM is larger than expected based on light scattering. Large MWP particles were heavier and adhered better to the SEM observation stub, while smaller MWP particles had a higher tendency not to adhere, even with subtle airflow during the sample preparation and measurement. Therefore, the SEM images in this study do not represent the real particle size distribution of MWP particles, but allow judging the shape and surface of MWP particles.

### 4.3.2 Viscosity of MWP dispersions and MWP o/w emulsions

Figure IV - 2 shows the viscosity-shear rate curves of MWP dispersions and MWP o/w emulsions. The viscosity of MWP dispersions increases with increasing MWP concentration from 0.5% to 8%, as would be expected for a colloidal suspension. MWP dispersions with concentrations lower than 4% displayed Newtonian flow behavior. The 8% MWP dispersion displayed slight shear thinning behavior and very slight hysteresis. This may be due to the formation of clusters at high MWP concentration. Similar flow behavior was observed for MWP o/w emulsions except that with increasing fat content the MWP o/w emulsions displayed higher viscosity and slightly stronger shear thinning behavior (See Appendix).

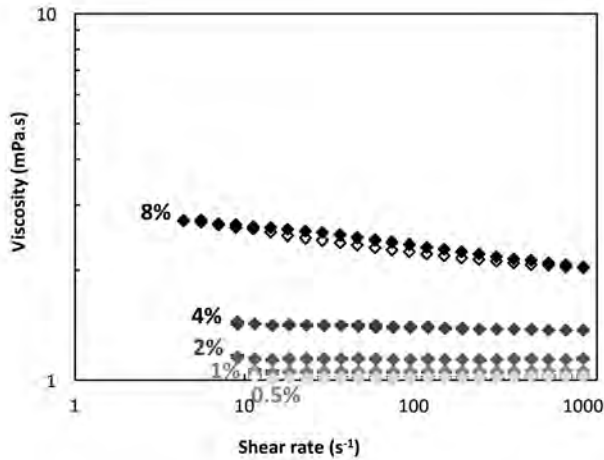
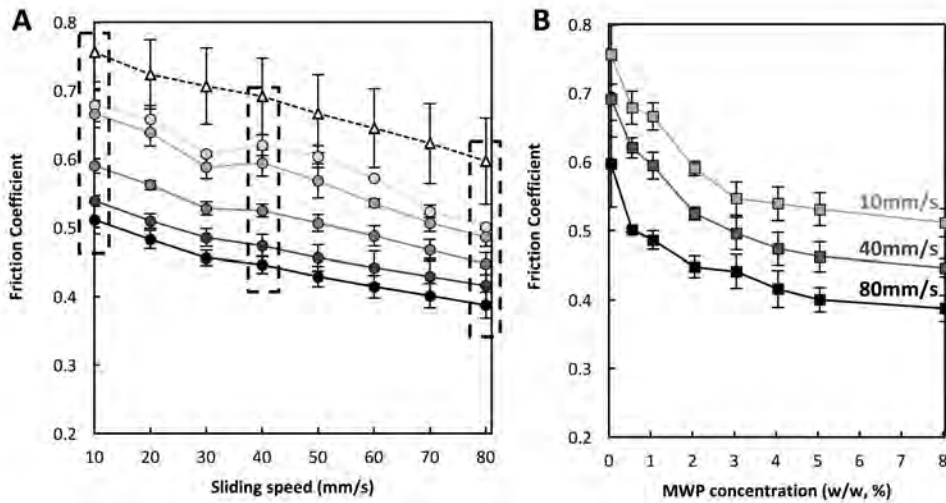


Figure IV - 2 Viscosity as function of increasing shear rate (closed symbol) and decreasing shear rate (open symbol) for MWP dispersions varying in concentrations from 0.5% to 8% (w/w).

### 4.3.3 Tribological properties of MWP dispersions

Figure IV - 3A shows the friction coefficient of MWP dispersions at different MWP concentrations as a function of sliding speed. We have several observations. First, for all concentrations the friction coefficient of MWP dispersions decreases with increasing sliding speed, indicating that all MWP dispersions at this condition are in the mixed lubrication regime of the Stribeck curves. No signs of a boundary lubrication regime are observed even at the lowest sliding speeds (10 mm/s). Chojnicka reported that for a WPI aggregates solution the transition from boundary to mixed regime was between 10 - 15 mm/s, using a ring on disk soft contact tribometer within a speed range from 5 - 600 mm/s (Chojnicka *et al.*, 2008). One reason that we did not observe the boundary regime might be that the starting speed

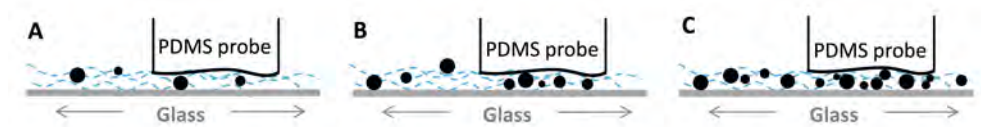
of our tribological measurements is not low enough. Another reason might be that in this study the size of MWP particles is much larger than the size of WPI aggregates (30 – 80 nm) studied by Chojnicka. The larger MWP particles might separate the tribo-surfaces better and therefore the boundary lubrication regime is not observed in our study.



**Figure IV - 3** Friction coefficient of MWP dispersions at various concentrations as a function of sliding speed.  $\triangle$  = 0% MWP (= water);  $\circ$  = 0.5% MWP;  $\bullet$  = 1% MWP;  $\circ$  = 2% MWP;  $\bullet$  = 4% MWP;  $\bullet$  = 8% MWP. B: Friction coefficient of MWP dispersions at different sliding speed as a function of MWP concentration (the same data as A, concentration at 3% and 5% are also shown), compared with 40% pork fat emulsion stabilized with 1% WPI.  $\blacksquare$  = MWP 10mm/s;  $\blacksquare$  = MWP 40mm/s;  $\blacksquare$  = MWP 80mm/s. Error bars represent the standard deviation of the triplicates.

Second, Figure IV - 3A shows that with increasing concentration of MWP from 0.5% to 8% the friction coefficient of the dispersions decreases. The smallest amount of MWP added to water (0.5%) reduced the friction coefficient compared to using only water by up to 16%. The lubrication ability of MWP in water can be explained by its spherical shape and its small particle size. The spherical particles can reduce the friction by reducing the contact area between the tribo-pair surfaces and changing the local relative motion from sliding to rolling (de Wijk *et al.*, 2005; Gabriele *et al.*, 2010). This is referred to as a ball bearing mechanism. We illustrated this mechanism in Scheme IV - 1. The small size may contribute to the entrainment of MWP particles in the contact region of the tribo-pair (Gabriele *et al.*, 2010). Although in our study there are particles that are already pre-entrained between the tribo-pair surfaces, the small size can ensure that the particles remain in between the surfaces.





**Scheme IV - 1** Schematic representation of behavior of MWP particles (black filled circles). A) low MWP concentration: limited number of MWP particles are in the contact zone; B) medium MWP concentration: number of MWP particles in contact zone increases forming a particle layer. C) high MWP concentration: number of MWP particles in contact zone increases further, but particle layer may become saturated.

It has been reported that whey protein particles introduced into the contact zone of a tribometer increased the friction coefficient, especially when particles were small (Chojnicka-Paszun *et al.*, 2014b). This is in contrast to what we observed. As a first reason for this apparent discrepancy we note that in our study the fluid that contained the MWP particles was water, while in Chojnicka's study WPI particles were dispersed in polysaccharides solutions. Since in both studies the mixed regime is relevant, the lubrication properties of different bulk solutions are likely to influence the behavior. As a second possible reason, we note that most of the particles (0.1 – 1  $\mu\text{m}$ ) in our study are much smaller than in the Chojnicka's study (8 – 57  $\mu\text{m}$ ). Indeed in another study of Chojnicka, the friction coefficient of globular protein aggregates with diameter of 30-80 nm decreased with increasing concentration (Chojnicka *et al.*, 2008). So, the size of particles that can reduce friction may have to be in a certain range relative to the tribo-pair used. The third possible reason might be that in different studies different methodologies (Joyner *et al.*, 2014a), and different surfaces were used (e.g. the surface hardness, surface hydrophobicity, and the ratio between the size of the particles and roughness of the surfaces) (Dresselhuys *et al.*, 2008d; Joyner *et al.*, 2014b; Ranc *et al.*, 2006).

To obtain a more detailed insight in the influence of the MWP concentration on the friction coefficient, results in Figure IV - 3A at speed of 10 mm/s, 40 mm/s and 80 mm/s are shown in Figure IV - 3B. This figure shows that the decrease of friction coefficient with increasing MWP becomes less pronounced when the MWP concentration is higher than 3%, and the friction coefficient seems to be reaching a plateau when the MWP concentration gets between 5% and 8%. Similar plateaus were observed by Garrec *et al.* (2013). They reported that increasing the volume fraction of kappa carrageenan particles from 0.3 to 0.6 would not decrease the friction in the boundary regime. The formation of a plateau was suggested to be due to the formation of particle layers by deposition of the particles (Garrec *et al.*, 2013). In our study we observed only the mixed regime, where a boundary film is indeed still important (Stokes *et al.*, 2013). Therefore particle layers could indeed be formed, and

influence the frictional behavior in our case. It is possible that the amount of particles could not be further increased due to the saturation of particles between the two tribo-pair surfaces, explaining why the friction coefficient could not be reduced further. It is also possible that with further increasing of particle concentration, the amount of particles that can enter and remain in the gap between the tribo-pair is restricted, therefore the friction coefficient could not be reduced further. We illustrated this possible explanation in Scheme IV - 1. A notable experimental fact is that the gradient of the friction reduction as a function of increasing concentration of MWP particles is independent of the sliding speed, for all particle concentrations (Figure IV - 3B).

We compared the friction coefficient of MWP dispersions with 40% fat-in-water emulsions which were stabilized by 1% WPI (data not shown). About 2 - 4% MWP in water showed equivalent friction coefficients as the 40% fat-in-water emulsion (sliding speed between 10 - 80 mm/s). This suggests that the MWP particles can effectively mimic the lubricity of fat droplets.

In the mixed regime observed in our study, the viscosity of the MWP dispersion is also relevant for lubrication. Chojnicka et al. tried to estimate the viscosity contribution to the friction coefficient of water solutions of WPI aggregates (Chojnicka *et al.*, 2008). They plotted Stribeck curves as a function of the product of entrainment speed and effective viscosity. They found that the friction curves of different concentration of WPI collapsed onto one master curve. They concluded that the decrease in friction coefficient with increasing WPI aggregates concentration is only due to the increase in viscosity. We investigated the Stribeck curve as a function of the product of viscosity and sliding speed divided by load (friction parameter) (Figure IV - 4).

We found that with increasing concentration of MWP dispersions due to the increasing viscosity, the friction curve shifted down and to the right. We still observe significant reduction of friction coefficient with increasing MWP concentration. We conclude that in addition to the effect of bulk viscosity on lubrication of the MWP dispersions, the MWP particles themselves contribute to lowering the friction. This remaining contribution of the MWP particles to reducing the friction is most probably caused by the ball-bearing effect.

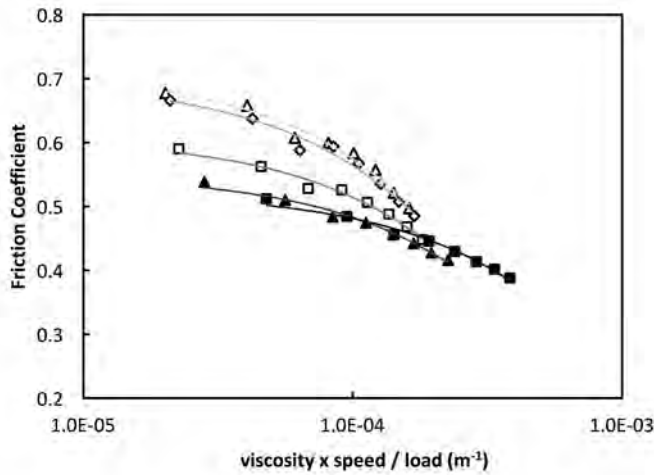
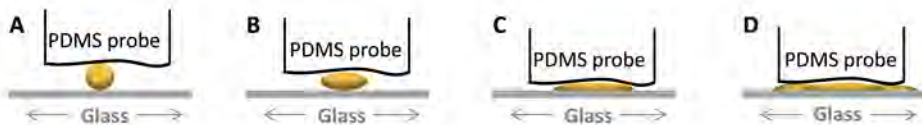


Figure IV - 4 Friction coefficient of MWP dispersions at various concentrations as a function of the product of viscosity and sliding speed divided by applied load (friction parameter).  $\triangle$  = 0.5%,  $\diamond$  = 1%,  $\square$  = 2%,  $\blacktriangle$  = 4%,  $\blacksquare$  = 8%.

#### 4.3.4 Tribological property of MWP o/w emulsions

Friction force of MWP o/w emulsions that are stabilized with either 1% WPI or 2% Tween 20 were measured as a function of speed. In Figure IV - 5 the friction curves of MWP o/w emulsions are shown as a function of the friction parameter. We observe that the friction curves of the MWP o/w emulsions display both the boundary and mixed regime of the Stribeck curves. The lubrication mechanism for o/w emulsion has been explained by the plate-out theory (Schmid & Wilson, 1996), and is illustrated in Scheme IV - 2. During the shear in the tribometer, the oil droplets deform and spread on the surface to form film patches covering the surface. These film patches lead to a decrease in friction.



Scheme IV - 2 Plate-out theory. A) Fat droplet (yellow) in between tribo-pair surfaces; B) Fat droplet deforms under shear; C) Fat droplet spreads over surface (plated out); D) Multiple plated fat droplets form fat film patches. (Adapted from (Schmid *et al.*, 1996))

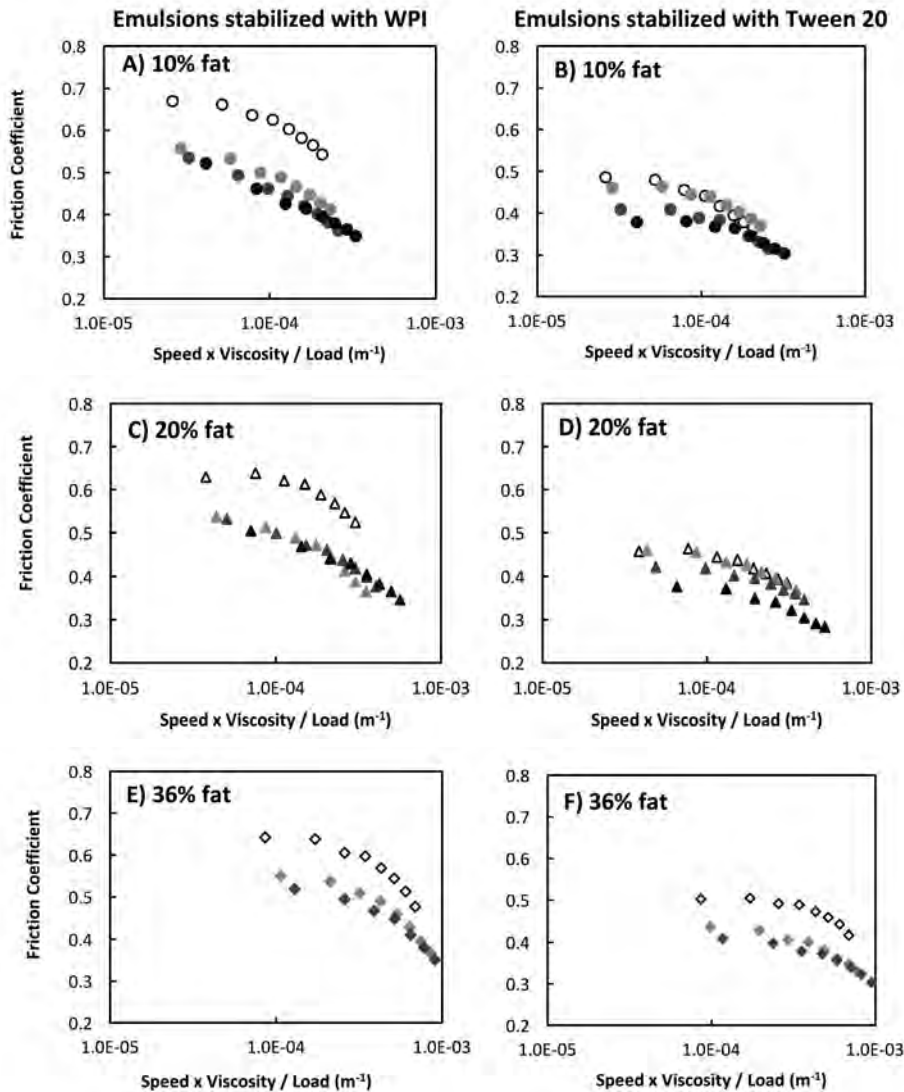
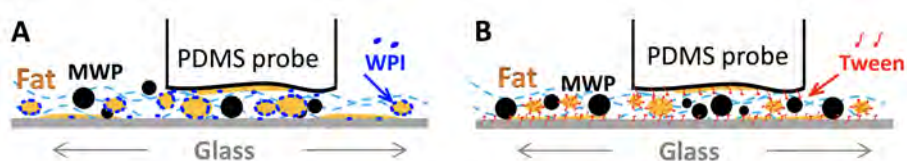


Figure IV- 5 Friction coefficient of MWP in emulsions at various concentrations as a function of the product of sliding speed and viscosity divided by load (friction parameter). Emulsions are pork fat emulsions stabilized either with 1% WPI (A, C and E), or with 2% Tween 20 (B, D and F). ● (A&B): 10% fat with 0%, 1%, 2%, 4% MWP; ▲ (C&D): 20% fat with 0%, 1%, 2%, 4% MWP; ◆ (E&F): 36% fat with 0%, 1%, 2% MWP. Increasing darkness of the symbol color indicates increasing MWP concentration.

Systems with droplets that are stabilized with Tween 20 display a more pronounced boundary regime (covering a longer range of the friction parameter) and have much lower friction coefficients than the systems with WPI stabilized droplets. One possible explanation

is that the boundary film may be formed less easily when droplets are stabilized with WPI compared to Tween 20. The formation of a boundary fat film is related to the adhesion and subsequent spreading of fat droplets on the surfaces of the tribo-pair. The sensitivity of adhesion and spreading of fat droplets on the surfaces determines friction (Dresselhuis *et al.*, 2008a). The adhesion and spreading of fat droplets is related to the amount and type of emulsifiers used (Bellamy *et al.*, 2009; Dresselhuis *et al.*, 2008a; Grosse & Estel, 2000). It is reported that protein-rich emulsions (1% WPI) have high mechanical stability against rupture of the droplets and therefore are less prone to spread on solid surfaces (Dresselhuis *et al.*, 2008d). Tween 20 is a non-ionic and small molecular weighted surfactant that allows to be more easily spread on the surfaces and form a boundary film, compared to the WPI stabilized droplets.

Another possible explanation could be that the boundary film is formed due to absorption of Tween 20 on the tribometer surfaces (Graca *et al.*, 2007; Schmid *et al.*, 1996). The surface can be covered with a monolayer of surfactant molecules that prevent the solid surfaces to have direct contact (Chen *et al.*, 2002; Spikes, 1993). A schematic representation is shown in Scheme IV - 3. Scheme IV - 3A shows a system that contains MWP particles and WPI stabilized emulsion droplets. In the contact zone of a tribometer, some of the fat droplets form a fat film (plating-out) due to the existence of the WPI emulsifier, while some of the intact fat droplets may still be able to enter the contact zone together with the MWP particles. MWP particles in the contact zone behave as ball-bearing. Scheme IV - 3B represents a system that contains MWP particles and Tween 20 stabilized emulsion droplets, and in this situation Tween 20 is able to absorb on the surface and form a robust separating layer (Graca *et al.*, 2007).



**Scheme IV - 3 Schematic representation of A) behavior of WPI stabilized o/w emulsions and MWP particles in contact zone of a tribometer; B) behavior of Tween 20 stabilized o/w emulsions and MWP particles in contact zone of a tribometer.**

Independent of the emulsifier used, with increasing fat content from 10% to 36%, due to the increased viscosity (Appendix), the friction curves shift to the right. However, the friction coefficient is not noticeably reduced with increasing fat content. According to the plate-out

theory, only a small additional amount of fat is needed to cover the surfaces and cause reduction of friction (Schmid *et al.*, 1996). With increasing concentration of MWP from 0 to 4%, the friction curves shifted not only to the right due to increasing viscosity, but also shifted downwards (similar to Figure IV - 4). This gives further evidence that MWP is able to reduce friction, presumably due to the ball-bearing mechanism. Similar to MWP in water (Figure IV - 3), the gradient of the reduction in friction coefficient decreases at higher MWP concentrations in the presence of emulsion droplets.

In addition, Figure IV - 5 shows that the friction reduction by MWP is more efficient than by fat. We notice that the magnitude of the friction reduction in Tween 20 stabilized emulsions is less than in WPI stabilized emulsions. We suggest that this is probably due to the effect of a boundary layer formed by Tween 20, which decreases the prominence of the ball bearing effect by the MWP particles. We conclude that the lubrication property of emulsions containing MWP is a combined effect of boundary layer formed by emulsifiers, a MWP ball bearing, and a lubricating film formed by plate-out fat. It seems that the emulsifier boundary layer and the ball bearing of MWP contribute more to the lubrication properties than the lubricating fat layer.

#### **4.3.5 Large deformation properties of MWP gelatin gels and MWP emulsion-filled gels**

The effect of addition of MWP to emulsion-filled gelatin gels on the mechanical properties was investigated using uniaxial compression test. Young's modulus of emulsion-filled gelatin gels containing 5% fat droplets as a function of MWP concentration is shown in Figure IV - 6A. When droplets are bound to the matrix (WPI as emulsifier), Young's modulus of the gels increases with increasing concentration of MWP. This indicates that the MWP particles are bound to the gelatin gel matrix and the modulus of MWP particles is higher than the modulus of the gelatin matrix. When fat droplets are unbound to the matrix (Tween 20 as emulsifier), Young's modulus initially decreases (at 1% MWP) and then increases with increasing MWP concentration. The existence of free Tween 20 molecules may obstruct the "bound" interaction between MWP and the gelatin matrix, therefore at low concentrations, MWP becomes partly unbound. This may explain why at low MWP concentration (i.e. 1%) the Young's modulus is decreased. With further increasing the MWP concentration, the MWP particles, which are bound to the matrix, dominate the mechanical properties and enhance the gel strength.

As previously reported (Liu *et al.*, 2015; Sala *et al.*, 2007a; van der Poel, 1958), the emulsion-filled gels containing unbound fat droplets reveal a lower Young's modulus than gels containing bound fat droplets. It was suggested that using unbound fat droplets could be a fat-reduction strategy to enhance perception of fat-related sensory attributes (Liu *et al.*, 2015). From the current study we suggest that the loss of Young's modulus due to the unbound droplets can be partially compensated by the addition of MWP. The combination of unbound droplets and MWP can control the Young's modulus.

In practical applications, for example cheese, it was reported that MWP particles behave as inert, (non-interacting, or unbound) fillers entrapped in the casein network (Schenkel *et al.*, 2011, 2013; Steffl *et al.*, 1999). The inert MWP particles weaken the casein network. Furthermore, the reported results of mechanical properties of cheeses that contain MWP among different studies are inconsistent (Schenkel *et al.*, 2013). This is different from our results. Therefore, one has to be aware that the behavior of MWP particles is not universal in different types of semi-solid food matrices. MWP can be bound or unbound to the matrix, depending on properties of the matrix and the additional emulsifiers.

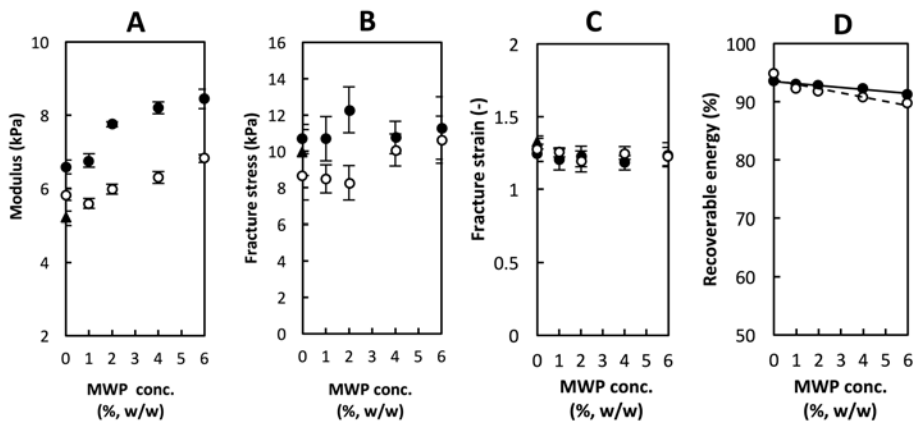


Figure IV - 6 Large deformation properties of 5% pork fat emulsion-filled gels containing MWP at different concentrations. A) Young's modulus; B) Fracture stress; C) Fracture strain; D) Recoverable energy. ▲ = 4% gelatin (no emulsion added to gelatin matrix); ● = bound fat droplets (1% WPI); ○ = unbound fat droplets (2% Tween 20). Error bars represent the standard deviation of 8 measurements.

Figure IV - 6B & C show the fracture stress and strain of the above-mentioned gels. The addition of MWP slightly increases the fracture stress of the gels when fat droplets are bound to the matrix. When fat droplets are unbound to the matrix, the fracture stress of gels first decreases and then increases again with increasing concentration of MWP. Independent of

the interaction between the gelatin matrix and fat droplets, the addition of MWP does not affect the fracture strain of the emulsion-filled gels.

Figure IV - 6D shows the recoverable energy of 5% emulsion-filled gels that contain MWP particles. With increasing MWP concentration, the recoverable energy decreases slightly. Since MWP particles are bound to the gelatin matrix, they participate in the gelatin network and consequently influence recoverable energy, but only slightly.

Figure IV - 7 shows the effect of fat content on the Young's modulus of emulsion-filled gelatin gels containing 1% MWP. In Figure IV - 7A, when fat droplets are bound to the matrix and in the absence of MWP, increasing fat content from 0 to 15% leads to an increase in gel modulus. This is in consonance with literature (Liu *et al.*, 2015; Sala *et al.*, 2007a; van der Poel, 1958). A similar trend can be observed in the presence of 1% MWP. MWP further increases the Young's modulus slightly (which is also observed in Figure IV - 6A). In Figure IV - 7B, when fat droplets are unbound to the matrix and in the absence of MWP, increasing fat content also leads to an increase of Young's modulus, but to a lesser extent compared to gels with bound fat droplets. This is due to a combined effect of unbound fat droplets and solid fat in the fat droplets (solid fat content is 36% at 20 °C) (Liu *et al.*, 2015). In the presence of 1% MWP, as we have observed in Figure IV - 6A that they behave as unbound filler, the Young's modulus is slightly lower than in gels without MWP at 5% fat. This effect becomes larger at higher fat content (15%).

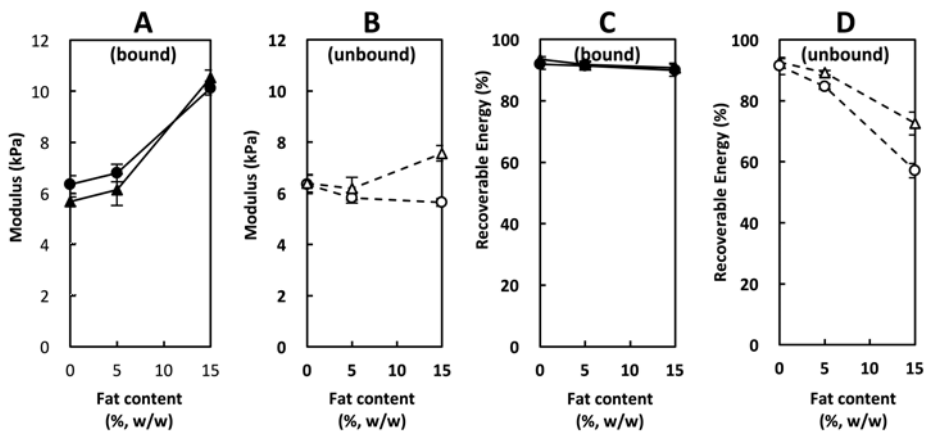


Figure IV - 7 Young's modulus (A & B) and recoverable energy (C & D) of emulsion-filled gelatin gels containing 1% MWP as a function of fat content. Filled symbol represents gels with bound fat droplets; empty symbol represents gels with unbound fat droplets; triangles represent 0% MWP; circles represent 1% MWP. Error bars represent the standard deviation of 8 measurements.



Figure IV - 7C & D show the recoverable energy of emulsion-filled gelatin gels containing 1% MWP as a function of fat content. When fat droplets are bound, regardless of the presence (1%) or absence of MWP, the recoverable energy of emulsion-filled gel is not influenced by increasing fat content. When fat droplets are unbound, the recoverable energy of emulsion-filled gelatin gels decreases with increasing fat content, and this effect is larger with the presence of 1% MWP (which behave as unbound fillers at low concentration).

#### **4.3.6 Tribological properties of MWP gelatin gels and emulsion-filled gelatin gels**

Figure IV - 8 shows the friction coefficient of 4% gelatin gels containing different concentrations of MWP. We observe similar tribological behavior of the MWP gelatin gels compared to aqueous MWP dispersions (Figure IV - 3). The friction coefficient of the gels decreases with increasing sliding speed. This suggests that the frictional behavior is in the mixed regime of the Stribeck curve. With increasing MWP concentration from 0 to 8%, the friction coefficient of gelatin gels decreases, similar to the behavior observed in liquid MWP dispersions. Furthermore, the friction coefficient of 4% gelatin without added MWP is smaller than water (Figure IV - 3). The magnitude of the friction reduction by MWP in gelatin is smaller than for water. Gelatin gels show lower friction because gelatin itself has a lubricating effect, probably due to the formation of a thin film on the tribo-pair surfaces (Dowson, 1993). Because the MWP particles are bound to the gelatin matrix, they could not be released easily from the matrix. Since the MWP particles are trapped within the gelatin matrix, the rolling of MWP is probably restricted, especially at lower concentrations. This could be the reason why the ball bearing effect of MWP in gelatin gels is less pronounced than in aqueous dispersions of MWP.

Figure IV - 9 shows the friction coefficient of emulsion-filled gels containing bound pork fat droplets and MWP particles. The friction curves stay in the mixed regime of the Stribeck curve. At low fat content (5%), addition of MWP decreased the friction coefficient of the emulsion-filled gelatin gels, similarly as for the gelatin gels without fat (Figure IV - 8). This indicates that the 5% bound fat droplets did not contribute much to the friction reduction. At relative higher fat content (15%), the magnitude of friction reduction by MWP is slightly bigger than at lower fat content, especially at lower speed. Although the fat droplets are bound to the matrix, the shear applied during the friction measurements might release fat and might provoke fat coalescence that facilitated lubrication. This could explain why at higher fat content the friction coefficients of the gels are lower.

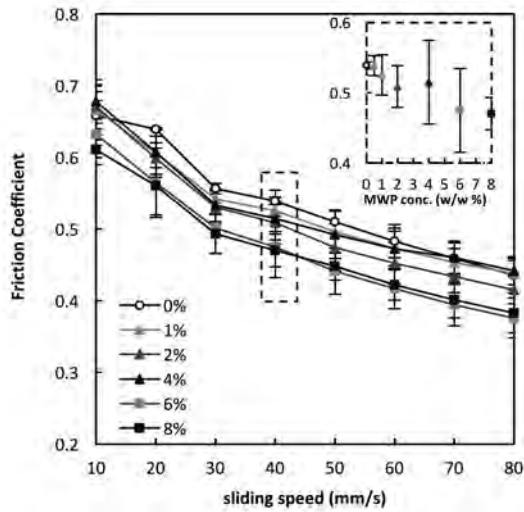


Figure IV - 8 Friction coefficient of 4% gelatin gels containing MWP as a function of sliding speed. Inner figure: friction coefficient of the gels as a function of MWP concentrations at 40mm/s. ○ = 0% MWP; ▲ = 1% MWP; ▲ = 2% MWP; ▲ = 4% MWP; ■ = 6% MWP; ■ = 8% MWP. Error bars represent the standard deviation of triplicate measurements.

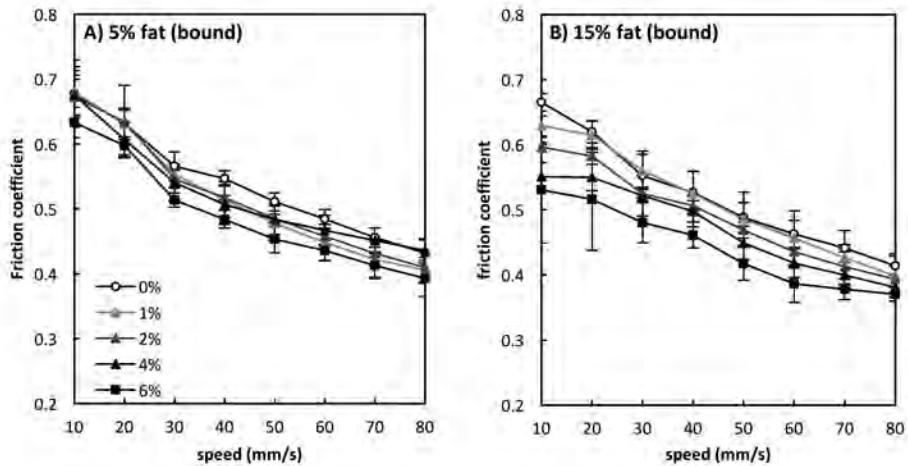


Figure IV - 9 Friction coefficient of emulsion-filled gels (droplets stabilized with 1% WPI) containing MWP as a function of sliding speed. ○ = 0% MWP; ▲ = 1% MWP; ▲ = 2% MWP; ▲ = 4% MWP; ■ = 6% MWP. Error bars represent the standard deviation of triplicate measurements.

Figure IV - 10 shows the friction coefficient of emulsion-filled gelatin gels containing unbound pork fat droplets and MWP particles. We expected that more fat could be released when fat droplets are unbound (Liu *et al.*, 2015). We indeed observed that gels with unbound fat droplets have lower friction coefficient than gels with bound droplets. The lower friction coefficient might also be attributed to the presence of surfactant (Tween 20), as discussed before. At low fat content (5%) the friction curves were in the mixed regime of the Stribeck curve, and the friction coefficient of the gels decreased with increasing MWP. At higher fat content (15%), however, this trend was not observed. At low sliding speed (10 mm/s), the friction coefficients were very low and were independent of the MWP concentration. With increasing speed from 10 to 30 mm/s, friction coefficients increased and started to decrease upon further increasing of sliding speed (from 40 to 80 mm/s). The intricate friction data at low speed is probably reflecting the large deformation properties of the gels. The gel containing 15% unbound droplets and MWP (esp. at 1%) had the lowest Young's modulus and recoverable energy (Figure IV - 7), therefore this gel (shown in Figure IV - 10B) broke down more easily and might have released fat more easily, than the gels that had higher Young's modulus. At higher speeds (> 30 mm/s) the friction coefficients of gels decreased with increasing MWP concentration until 4%. Further increase of MWP concentration to 6% did not exert a better lubrication.

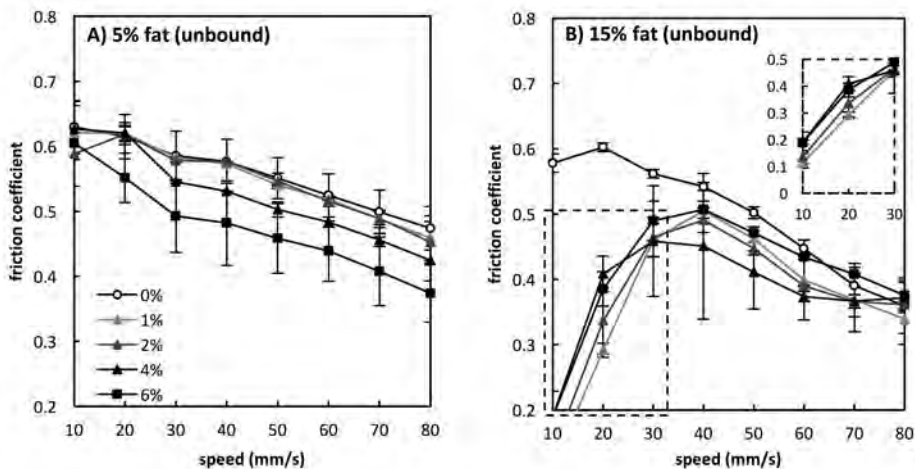


Figure IV - 10 Friction coefficient of emulsion-filled gels (droplets stabilized with 2% Tween 20) containing MWP as a function of sliding speed. ○ = 0% MWP; ▲ = 1% MWP; ▲ = 2% MWP; ▲ = 4% MWP; ■ = 6% MWP. Error bars represent the standard deviation of triplicate measurements.

## 4.4 Conclusions

We investigated the rheological and tribological properties of MWP particles as a fat replacer in liquid and semi-solid model foods. We conclude that the addition of MWP effectively reduces the friction coefficient for water and o/w emulsions. We demonstrated that the reduction of friction coefficient by MWP is not only caused by an increase of viscosity. Our results provide strong evidence that the friction reduction of MWP in liquids is mainly due to ball-bearing lubrication.

The addition of MWP to semi-solid model gels also decreased the friction coefficient, but to a smaller extent compared to the liquids. The mechanism underlying the lubrication behavior of multi-component semi-solid foods that contain fat droplets, emulsifier, fat replacers such as MWP embedded in a gel matrix is complex. We conclude that different components affect the lubrication properties of the composite foods through different mechanisms. Fat droplets mostly reduce friction due to the formation of fat film patches following a plate-out mechanism. Emulsifiers influence the formation of an emulsifier layer through adhesion on the respective surfaces, whereas MWP reduces friction due to a ball-bearing mechanism. In addition, the influence of bulk viscosity, gel fracture behavior, and the interactions between the MWP and its surrounding matrix and emulsifiers, need to be accounted for.

The ball bearing lubrication of MWP particles reveals possibilities of using other food colloidal micro-particles that may have similar ball-bearing lubrication properties, as fat replacers. For future studies we also recommend to inter-relate the tribological, rheological and sensory properties of the multicomponent foods. This will allow developing and selecting suitable fat replacers for specific food products with required textural and sensorial properties.

## 4.5 Appendix

Viscosity of MWP o/w emulsions

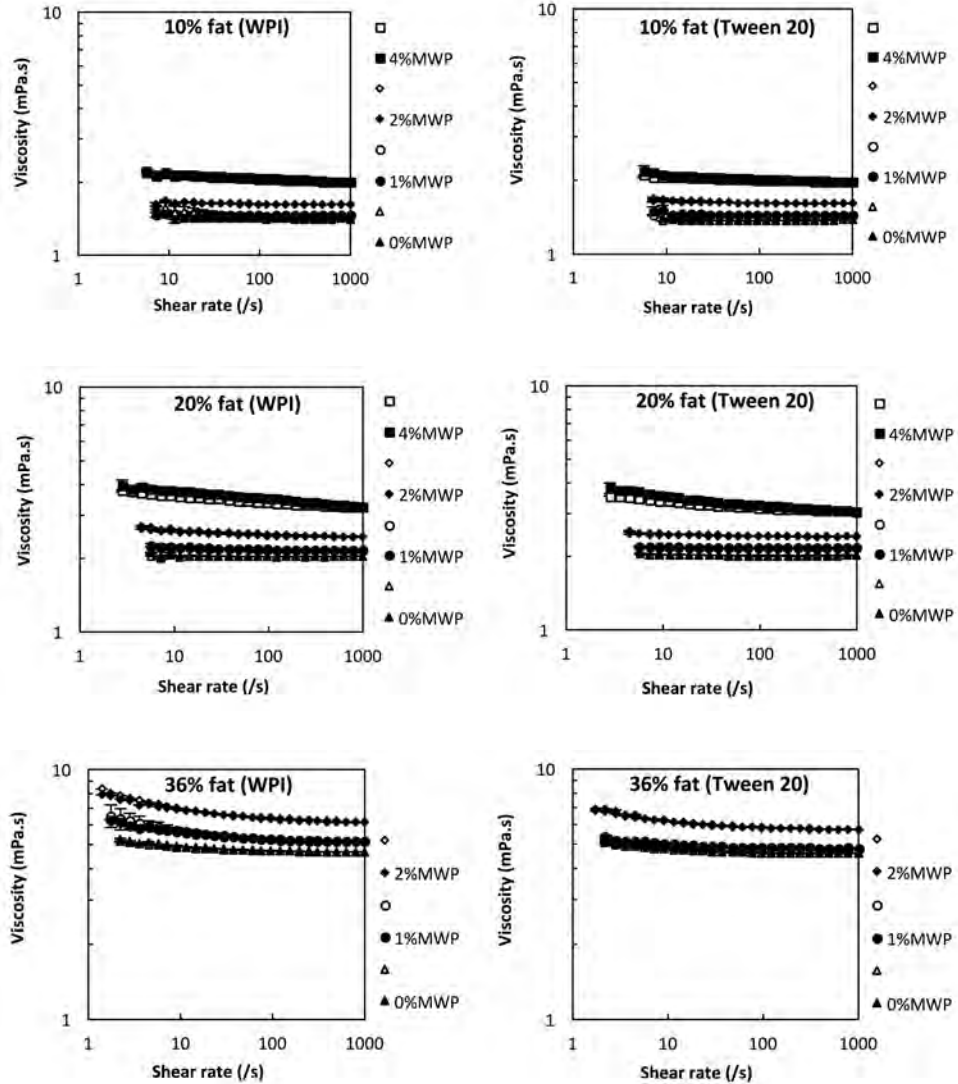
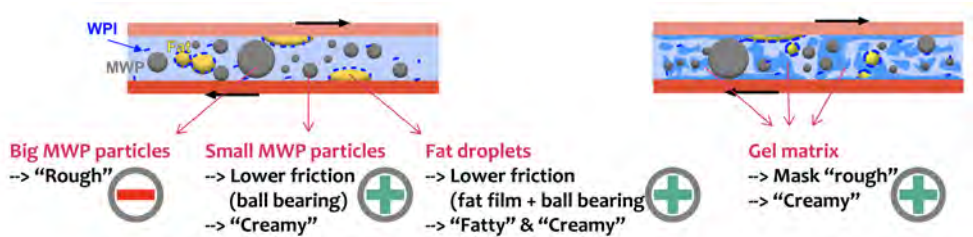


Figure IV - A 1 Viscosity as function of increasing shear rate (closed symbol) and decreasing shear rate (open symbol) for MWP o/w emulsions at different concentrations.



# Chapter 5

## Effect of microparticulated whey protein on sensory properties of liquid and semi-solid model foods



**Submitted as:**

**Kun Liu, Markus Stieger, Erik van der Linden, Fred van de Velde, Effect of microparticulated whey protein on sensory properties of liquid and semi-solid model foods.**

## Abstract

This work describes the sensory properties of microparticulated whey protein (MWP) particles in relation to their rheological and tribological properties. The aim of this work is to obtain a better understanding of the sensory perception of MWP particles compared to oil droplets in two food matrices. We used liquid MWP-o/w emulsions with controlled viscosities and semi-solid MWP-emulsion-filled gelatin gels as food model systems.

Consistent with our previous findings, MWP showed good lubrication properties due to ball bearing mechanism in both liquid and semi-solid systems as measured by tribology. Sensory results (QDA) revealed that small MWP particles (smaller than the size corresponding to the detection threshold) contributed to perception of creaminess due to their lubrication property. Big MWP particles (bigger than the size corresponding to the detection threshold) contributed to the rough and powdery perception, and thus suppressed perception of creaminess. MWP particles did not contribute to perception of fattiness in contrast to oil droplets. The perception of fattiness was probably related to the film formation properties of oil. As a result, MWP in liquid emulsions were generally perceived as rough but not creamy. In the case of MWP-emulsion-filled gels, although the gel matrix restrained the lubrication function of MWP particles, it also masked the rough perception of big MWP particles. Due to the combined effect of both oil droplets and MWP particles, MWP in gels resulted in an overall positive effect on the creamy perception.

As a conclusion, MWP contributes to fat-related sensations in a different way than oil does. The perception of MWP particles is related to the size of the particle as well as the properties of the surrounding matrix. Thus, it is of great importance to consider the effect of the food matrix when apply particle fat replacers in foods.



## 5.1 Introduction

Microparticulated whey protein (MWP) has been introduced as a texture modifier and fat replacer in many types of foods including thickened emulsions (Chung *et al.*, 2014), mayonnaises (Tamime *et al.*, 1995), yoghurts (Tamime *et al.*, 1995; Torres *et al.*, 2009; Torres *et al.*, 2011), ice creams (Yilsay *et al.*, 2006), cheeses (McMahon *et al.*, 1996; Sahan *et al.*, 2008; Singer, 1996; Sturaro *et al.*, 2015) and emulsion-filled gels (Liu *et al.*, 2016). MWP particles are manufactured from whey protein concentrate through different methods (Cheftel *et al.*, 1993; Ritzoulis *et al.*, 2015). The fat mimicking functionality of MWP has been mainly attributed to the particles' spherical shape and small size (typically < 5  $\mu\text{m}$ ), which is comparable to that of oil droplets in food emulsions. MWP particles in aqueous phase have been suggested to roll freely over one another under applied shear, which has been suggested to be responsible for a creamy and smooth mouthfeel perception (Cheftel *et al.*, 1993; Kilcast *et al.*, 2002; Singer, 1996). Our previous study showed evidence for such ball-bearing lubrication properties of MWP in liquid and semi-solid food matrices (Liu *et al.*, 2016). However, the extent by which MWP can impart fat-related sensory perceptions and the contribution of MWP lubrication properties to fat-related sensations is not well understood. We note that although MWP particles can fulfill the friction reduction function of oil droplets, the underlying mechanisms for this might be considerably different from oil droplets. MWP can reduce friction in foods by a ball-bearing mechanism, while oil droplets can be deformed and coalesce with adjacent oil droplets to form an oil film that reduces friction (Schmid *et al.*, 1996). The difference in mechanisms underlying the reduction in friction may induce differences in sensory perception of fat-related attributes, such as creaminess.

Creaminess is a complex sensory attribute that is considered as one of the most appreciated fat-related sensations. Creaminess is related to multiple food properties. Several pivotal studies have suggested that creaminess can be predicted from thickness, smoothness and slipperiness, which are mainly related to the viscosity of the foods and their lubrication properties in the mouth (Cussler *et al.*, 1979; Kokini, 1987; Kokini *et al.*, 1983). Melting of foods in mouth can also influence perception of creaminess, which might be related to the lubrication effect of the molten layer (Kokini, 1987). Apart from these factors, the presence of particles also plays an important role in creaminess perception (Kilcast *et al.*, 2002; Krzeminski *et al.*, 2013). Large and non-smooth particles added to foods are often associated with several unappreciated sensory attributes, such as roughness, dryness, grittiness and powdery (Cheftel *et al.*, 1993; Krzeminski *et al.*, 2013; Petersson *et al.*, 2013). Perception of these sensory attributes might suppress creaminess perception (de Wijk *et al.*, 2006a;

Singer, 1996; Wood, 1974). In contrast, when the particles are small, soft, and are able to roll over each other, they contribute to creaminess perception. However, when the particles are too small, they are thought to be unable to provide a so-called “substantialness” impression, and might instead contribute to “watery” perception (Singer, 1996).

As we have outlined, the sensory perception of particles can be associated with roughness, dryness, grittiness, and powdery. The perception of these attributes results from the friction between the particles and the oral mucosa (de Wijk *et al.*, 2005; Hollins *et al.*, 2000). The detection of particles in mouth is related to the size, concentration, shape, and hardness of the particles, properties of the matrix in which the particles are dispersed, interactions between particles and matrix, as well as interactions of the particles with oral surfaces and saliva (de Wijk *et al.*, 2005, 2006a; Engelen *et al.*, 2005a; Engelen *et al.*, 2005b; Heath *et al.*, 1999; Imai *et al.*, 1995; Kilcast *et al.*, 2002; Minifie, 2012; Petersson *et al.*, 2013; Sala *et al.*, 2015; Tyle, 1993). Therefore, the particle size threshold for perception of particles embedded in a food matrix may depend on all factors mentioned above. For example, hard particles with irregular shape may already be perceived at low concentrations even when their size is very small, while soft and smooth particles may not be easily detected at a larger size range (de Wijk *et al.*, 2005; Engelen *et al.*, 2005b; Tyle, 1993). Despite the fact that MWP is often found to give a creamy and smooth mouthfeel, there is rarely any information available regarding the other potential particle-related perceptions of MWP. In addition, the effect of the rheological properties of the matrix in which particles are dispersed on sensory perception is not well understood. Engelen *et al.* (2005b) observed that the viscosity of the liquid phase did not influence perceived particle size of SiO<sub>2</sub> and polystyrene spheres. They ascribed this finding to the fact that they only investigated two viscosities that were both adequately high to disguise particles. Petersson *et al.* (2013) found that in the case of starch gels, the viscosity of the dispersed phases did not influence the detection threshold of rye bran particles. They suggested that saliva dilution and enzymatic breakdown of the foods is more important for sensory perception than the effect of viscosity prior to ingestion. Imai *et al.* (1995) observed that the perceived grittiness of microcrystalline cellulose decreased as the viscosity of the dispersed phase increased. One may notice that the particles in these references are all different. In the case of MWP, most literature on the perception of MWP particles is limited to their applications in thick foods, such as yoghurts, cheeses or semi-solid emulsions, whereas to our knowledge sensory perception of MWP in thin, liquid foods has not been reported.

Consequently, by investigating the sensory properties of MWP particles in relation to their rheological and tribological properties in liquid and semi-solid foods, we aim for a better

understanding of the sensory perception of MWP particles compared to oil droplets in different food matrices. We also aim to clarify the relationships between different sensory attributes, such as roughness, fattiness and creaminess. In this study, we used liquid MWP-o/w emulsions and semi-solid MWP-emulsion-filled gelatin gels as food model systems. In order to study the influence of viscosity on perceived creaminess, liquid MWP-o/w emulsions were designed to have two sub-groups of iso-viscosity.

## **5.2 Materials and methods**

### **5.2.1 Materials**

Microparticulated whey protein (MWP) (Simplese® 100) was provided by CPKelco (Lille Skensved, Denmark). Powdered whey protein isolate (WPI, Bipro™) was obtained from Davisco International Inc. (La Sueur, MN, USA). Sunflower oil was bought from a local supermarket (Wageningen, the Netherlands). Porcine skin gelatin (bloom value 240 - 260) was provided by Rousselot (Gent, Belgium). All materials were used without further purification. All samples for sensory evaluation were prepared under food grade conditions. All samples were prepared with tap water.

### **5.2.2 Sample preparations**

#### **5.2.2.1 Preparation of MWP-o/w emulsions**

An aqueous phase containing 1% (w/w) whey protein isolate (WPI) as emulsifier was prepared. A stock o/w emulsion was prepared by mixing 40% (w/w) sunflower oil and 60% (w/w) aqueous phase. The mixture was pre-homogenized using an Ultra Turrax Polytron (Kinematica AG, Switzerland) for 2 min, and then homogenized at 310 bar (wherein the second stage was 30 bar) using a lab homogenizer Ariete (NS1001L-Panda, Niro Soavi, Parma, Italy).

A stock MWP dispersion 20% (w/w) was prepared at room temperature by dispersing MWP powder in water under stirring for 2 h. MWP-o/w emulsions were prepared by mixing the stock o/w emulsion, stock MWP dispersion and water at calculated ratios. The ratio of mixing was calculated to yield the compositions listed in Table V - 1. These emulsions were divided as two sub-groups based on their viscosity values at 50 s<sup>-1</sup>. Within each sub-group their viscosities are comparable.

All MWP-o/w emulsions were freshly prepared on the day of the physical measurements and stirred until the moment of further analysis to minimize sedimentation of MWP particles in the emulsions. For sensory evaluations, 20 g of the emulsion were filled in 30 mL plastic cups. All samples were prepared one day before sensory evaluation. These samples were stored in the refrigerator at 4°C, and kept at 20°C prior to sensory evaluation. The samples were gently shaken by the panelists prior to sensory evaluation to avoid sedimentation.

**Table V - 1 Overview of composition of all samples.**

	Sample code <sup>1</sup>	Oil (% w/w)	MWP (% w/w)	Gelatin (% w/w)	Viscosity at 50 s <sup>-1</sup> (mPa·s)	Average viscosity of the sub-groups (mPa·s)
Liquid (MWP-o/w emulsion) (two iso-viscous groups)	20-0	20	0	-	2.5 ± 0.1	2.3 ± 0.2
	10-4	10	4	-	2.2 ± 0.0	
	5-5	5	5	-	2.1 ± 0.0	
	0-7	0	7	-	2.2 ± 0.0	
	13-0	13	0	-	1.9 ± 0.1	1.7 ± 0.2
	9.5-1	9.5	1	-	1.6 ± 0.1	
	7-2	7	2	-	1.5 ± 0.0	
	5-2.8	5	2.8	-	1.6 ± 0.0	
Gel (MWP-emulsion-filled gel)	5-0	5	0	4	-	
	5-1	5	1	4	-	
	5-2	5	2	4	-	
	5-4	5	4	4	-	
	15-0	15	0	4	-	
	15-1	15	1	4	-	
	15-2	15	2	4	-	
	15-4	15	4	4	-	
	10-0	10	0	4	-	
	20-0	20	0	4	-	

<sup>1</sup> The numbers in the sample code denote oil content followed by MWP content.

### 5.2.2.2 Preparation of MWP-emulsion-filled gelatin gels

The previously described 20% (w/w) stock MWP dispersion and 40% (w/w) stock emulsion were mixed with gelatin solutions at different ratios to yield the MWP-emulsion filled gels listed in Table V - 1. The final concentration of gelatin in the aqueous phase was 4% (w/w). Prior to mixing with MWP, gelatin was first hydrated in water for 2 h at room temperature. Then gelatin was molten by heating at 80°C for 30 min in a water bath and was cooled

to 28°C under running tap water. After mixing gelatin with the MWP dispersion (that was prepared at room temperature), the mixtures were stirred for 10 min and cooled to 25°C before mixing with emulsions. For physical characterizations, the mixtures were filled in 60 mL plastic syringes. For sensory evaluations, fifteen grams of gel mixtures were filled in 30 mL sensory cups. The samples were stored in the refrigerator at 4°C for 20 h, and then kept at 20°C prior to analysis or sensory evaluation.

### **5.2.3 Sample characterization**

#### 5.2.3.1 Characterization of oil droplet and particle size

The droplet size distribution of stock o/w emulsions and the particle size of MWP dispersions were measured using a MasterSizer2000 (Malvern Instruments Ltd., Malvern, UK). The stock o/w emulsions and MWP dispersions were diluted 5 times with water. Samples were added into the measurement chamber until an obscuration rate of around 12% was obtained. A refractive index of 1.48, 1.47, and 1.33 was used for the oil droplet, MWP and the aqueous phase, respectively. For both oil droplet and MWP, an absorption index of 0.01 was used. The calculation of oil droplet and MWP particle size distribution was based on a general-purpose model. Volume-to-surface diameter ( $d_{3,2}$ ) was used as the parameter characterizing mean droplet size or particle size.

#### 5.2.3.2 Viscosity of MWP-o/w emulsions

The flow curves of all MWP-o/w emulsions were determined using a rheometer (Physica MCR 501, Anton Paar, Graz, Austria) at 20°C. The concentric double gap cylinder geometry was used. Continuous flow measurements were performed by increasing the shear rate in logarithmic steps from 10 s<sup>-1</sup> to 1000 s<sup>-1</sup> and then decreasing from 1000 s<sup>-1</sup> to 10 s<sup>-1</sup>. Average viscosity at 50 s<sup>-1</sup> was determined from the flow curves. Each sample was measured in duplicate.

#### 5.2.3.3 Characterization of large deformation properties of MWP-emulsion-filled gelatin gels

An Instron universal testing machine (M5543, Instron International Ltd., Belgium) equipped with plate–plate geometry was used to perform uni-axial compression tests on MWP-emulsion-filled gelatin gels at 20°C. The diameter of the cylindrical gel was 26.4 mm and the height of the specimen was 25 mm. A thin layer of paraffin oil was applied on both the top and bottom surface of the samples to prevent friction between sample and plate during compression. For the determination of Young's modulus, fracture stress and fracture strain, measurements were

performed at a constant compression speed of 1 mm/s up to a compression strain of 80%. For the determination of recoverable energy, measurements were performed at a constant compression and decompression speed of 1 mm/s up to a compression strain of 25% (Liu *et al.*, 2015). All measurements were conducted on six to eight specimens and the average values were reported.

#### 5.2.3.4 Characterization of tribological properties

The tribological properties of all samples were determined using a tribometer according to the method reported previously (Liu *et al.*, 2015). A fixed amount of emulsion (200  $\mu$ L) or an intact piece of gel (around 200 mg) was sheared in the tribometer between two surfaces. In this study, the gel pieces were not pre-sheared prior to the tribological measurement. The lower surface of the tribo-pair was made of glass and the upper surface of a flat-bottom PDMS probe. The load applied between the upper surface and the sample was 0.5 N. The glass surface was oscillated over a distance of 16 mm at an increasing oscillating speed from 10 mm/s to 80 mm/s with incremental steps of 10 mm/s. Each speed was kept for 10 oscillation cycles. The friction force ( $F_x$ ) was measured during the movement of the glass surface. Friction force at each speed was reported as the average value of 10 cycles. Prior to each measurement, the glass surface was cleaned with ethanol and water. All measurements were conducted in triplicate. Each measurement was performed at 20°C with a new PDMS probe.

#### 5.2.3.5 Paired comparison tests of MWP-o/w emulsions

For the two-alternative-forced-choice (2-AFC) paired comparison tests of MWP-o/w emulsions,  $n = 55$  naive participants were recruited. The average age was  $25 \pm 3$  years and 32 were females. Most participants were students from Wageningen University. All participants were healthy and non-smokers. After reading detailed information about the study, all participants gave written informed consent before the tests. All participants received a financial compensation after completion of the tests. All participants were instructed not to eat half an hour before the tests and not to drink caffeinated beverages two hours prior to the tests.

Three series of 2-AFC tests were performed to assess three sensory attributes: creaminess, sweetness and thickness. Within each series, one sensory attribute was assessed for each paired comparison. In each pair, subjects had to indicate which sample is more intensive with respect to the attribute. Definitions of the attributes and instructions were given to the participants.

Eight MWP-o/w emulsions divided into two sub-groups were tested (Table V - 1). Each sub-group contained four MWP-o/w emulsions with comparable viscosity. Within each sub-group, all four samples were compared with each other, yielding six pairs for each sub-group (Table V - 2). Within each pair, the ratio of the difference in oil content ( $\Delta\text{oil}$ ) and the difference in MWP content ( $\Delta\text{MWP}$ ) was calculated (Table V - 2).

**Table V - 2 Overview of samples used in the 2-AFC tests. Difference in oil and MWP content between the samples of each pair are listed together with ratio of difference in oil content and difference in MWP content (% w/w : % w/w).**

Average Viscosity (mPa·s)	Pair	Difference in oil content ( $\Delta\text{oil}$ )	Difference in MWP content ( $\Delta\text{MWP}$ )	Mass ratio of $\Delta\text{oil} : \Delta\text{MWP}$
2.3 ± 0.2	10-4/20-0	10	4	2.5
	5-5/20-0	15	5	3
	0-7/20-0	20	7	2.9
	5-5/10-4	5	1	5
	0-7/10-4	10	3	3.3
	0-7/5-5	5	2	2.5
1.7 ± 0.2	9.5-1/13-0	3.5	1	3.5
	7-2/13-0	6	2	3
	5-2.8/13-0	8	2.8	2.9
	7-2/9.5-1	2.5	1	2.5
	5-2.8/9.5-1	4.5	1.8	2.5
	5-2.8/7-2	2	0.8	2.5

Paired samples were randomized over participants. The presentation order within the pairs was balanced over participants (equal number of participants receiving either sample A or B first). Each sample was coded with a unique 3-digit random number. All participants attended two sessions on two days. In each session, six pairs of samples from the same sub-group were evaluated. Each session lasted approximately 30 min. The tasting sessions were carried out in meeting rooms in the university with good ventilation and comfortable light and temperature. Sensory booths were constructed using wooded planks to separate the participants. White bread, water and napkins were provided during the tasting sessions. Between samples within a pair, participants were instructed to rinse their mouth with water. Among samples from different pairs, participants were instructed to rinse their mouth with water and to eat a small piece of bread. Participants were allowed to swallow or expectorate the samples according to their preference. All samples were served at room temperature, and were shaken gently by the panelists prior to sensory evaluation to ensure homogeneity of all samples.

In order to familiarize the participants with the samples, two warm-up samples were presented to the participants prior to the paired-comparison tasting sessions. From each sub-group one sample was selected as the warm-up sample. Warm-up samples were not rated by the participants. In each session, after the warm-up samples, all six pairs were presented to each participant at the same time in an aluminum box. Participants were instructed to taste the paired samples in a given sequence. Each sample could be tasted multiple times. The participants were asked to identify the sample that was perceived as creamier, sweeter or thicker by writing the sample code on the questionnaire.

#### 5.2.3.6 Quantitative descriptive analysis (QDA) of MWP-o/w emulsions and MWP-emulsion-filled gelatin gels

##### Panel

The sensory properties of 8 emulsions and 10 emulsion-filled gels (Table V - 1) were evaluated by a trained sensory panel ( $n = 12$ ) in two separate studies according to the principles of Quantitative Descriptive Analysis (QDA) (Stone *et al.*, 2004). There was a two-week break between the QDA study of MWP-o/w-emulsions and the QDA study of MWP-emulsion-filled gelatin gels. The emulsion-panel consisted of 11 females and 1 male, with an average age of  $48.3 \pm 13.8$  years. Nine of the panelists of the emulsion-panel also participated in the gel-panel. The gel-panel was completed with 3 additional panelists. This panel also consisted of 11 females and 1 male, with an average age of  $45.2 \pm 15.9$  years. All panelists had extensive experience in QDA profiling of o/w emulsions and semi-solid food gels from several previous studies on similar emulsions and emulsion-filled gels. For these two studies, all panelists were trained in the description of taste, mouthfeel, aftertaste and after-feel attributes of the emulsions and emulsion-filled gels. All panelists gave written consent before the tests, and received a financial compensation after completion of the tests. This study did not require ethical approval by the local medical ethics committee under Dutch regulations. This study was conducted in line with the declaration of Helsinki.

##### Training and profiling sessions

The emulsion- and gel studies each consisted of six training sessions and two profiling sessions. Each session lasted for about 1 to 1.5 h. Training sessions were conducted to generate the attributes to reach consensus on their definitions, and to define the instructions to evaluate these attributes. In total 18 attributes were generated to describe the MWP-o/w emulsions and 22 attributes were generated to describe the MWP-emulsion-filled gels. The attributes were generated in Dutch and translated into English for this paper. After the



training sessions, two 1 h profiling sessions were conducted in sensory booths at 21°C with appropriate ventilation. The evaluation of the emulsions took place under red light to cover some slight color differences.

Each panelist received the samples in a different balanced random order so that context effects were levelled out. All products were labelled with 3-digit codes. All samples were evaluated in triplicate by all 12 panelists during the profiling sessions. The panelists evaluated the samples at individual speed by descriptive analyses on an unstructured 100 mm line scale with intensity ratings ranging from very little at 10% and very much at 90% of the line scale. All data were registered using EyeQuestion (Version 3.15.10, Logic8).

#### Tasting procedure

Regarding emulsion samples, panelists took multiple sips of each product. Prior to tasting each sample, panelists first shake the sample thoroughly to avoid sedimentation of particles. Taste attributes were evaluated first, followed by mouthfeel. A new sip was taken, just before assessment of the aftertaste and afterfeel attributes. After spitting out this sip, the aftertaste and afterfeel attributes were evaluated.

Regarding gel samples, panelists were instructed to pick up the sensory cups at the rim to prevent the product to be heated by the fingers. They took a standardized half-spoonful of the product to evaluate mouthfeel. After that, taste attributes were assessed with a new half-spoonful sample. After spitting out the samples, afterfeel and aftertaste attributes were evaluated.

Panelists cleaned their mouth with water and crackers between samples. During the evaluation, panelists could take tap water ad libitum.

#### 5.2.3.7 Statistical data analysis

For the 2-AFC tests, binomial tests were carried out for each pair using SPSS statistic software (IBM SPSS, V20). The proportions of participants choosing one sample over the other (k%) were used to present the results. A significance level of  $p < 0.05$  was used.

For the QDA, panel performance was checked with Senpaq (V3.2, 2007). Mean and standard errors were calculated (IBM SPSS, V21). Univariate analysis of variance (UNI-ANOVA) (IBM SPSS, V21) using a general linear model (GLM) was performed to analyze the effect of sample on intensity of all sensory attributes. Sample type was used as the fixed factor, panelists and triplicate sessions were used as random factors. Tukey's honest significant difference

(Tukey's HSD) test was performed as a post hoc analysis. In this test a significance level of  $p < 0.05$  was chosen. Principal component analysis (PCA) (IBM SPSS, V21) was used to analyze the relationships between sensory attributes of samples.

## 5.3 Results and discussions

### 5.3.1 Liquid samples: MWP-o/w emulsions

#### 5.3.1.1 Droplet and particle size

MWP dispersions had a bi-modal particle size distribution based on volume frequency with two peaks at  $0.4 \mu\text{m}$  and  $7.0 \mu\text{m}$ . This was similar to the particle size distribution reported previously (Liu *et al.*, 2016). The mean droplet size of the stock o/w emulsion was  $1.2 \pm 0.1 \mu\text{m}$ .

#### 5.3.1.2 Tribology

Figure V - 1A shows the friction coefficient of MWP-o/w emulsions with  $\eta = 2.3 \text{ mPa}\cdot\text{s}$  as a function of sliding speed. The 20% oil emulsion without MWP (20-0) showed the highest friction coefficient. A 10% oil emulsion with 4% MWP (10-4) showed significant lower friction coefficient although the oil content is 50% less than the 20% oil emulsion. This confirmed a previous finding that MWP particles have good lubricating characteristics (Liu *et al.*, 2016). With further increasing MWP concentration and lowering oil content, the friction coefficients did not decrease further. This was explained by the lack of oil lubrication and saturation of MWP particles in between the two surfaces in our previous study (Liu *et al.*, 2016).

Figure V - 1B shows the friction coefficient of MWP-o/w emulsions with  $\eta = 1.7 \text{ mPa}\cdot\text{s}$  as a function of sliding speed. With increasing concentration of MWP and thus decreasing oil content, friction coefficient decreased gradually. This confirmed our finding that MWP particles have good lubrication characteristics also in reduced-oil systems.

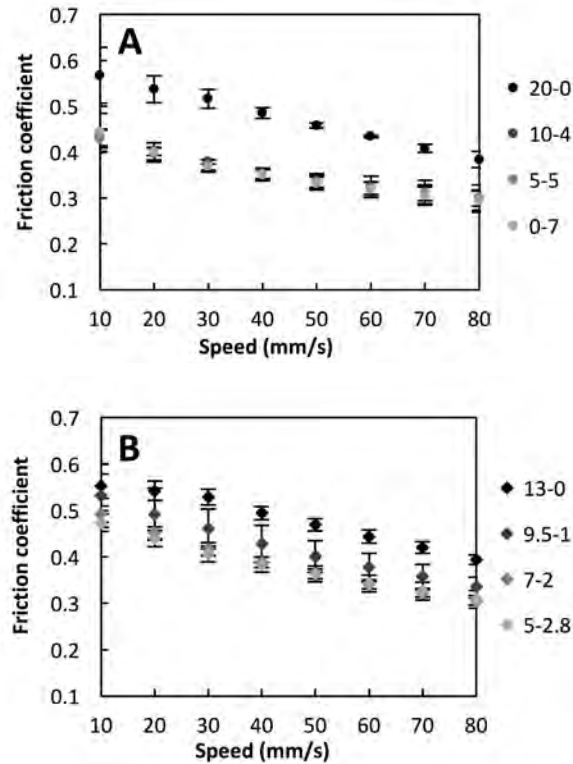


Figure V - 1 Friction coefficients of MWP-o/w emulsions. A) MWP-o/w emulsions with  $\eta = 2.3$  mPa·s; B) MWP-o/w emulsions with  $\eta = 1.7$  mPa·s. Error bars represent the standard deviation of triplicate measurements.

### 5.3.1.3 Paired comparison sensory tests

Figure V - 2 shows the proportions of panelists who rated the sample that contained more MWP in each pair as more intensive in (A) sweetness, (B) thickness or (C) creaminess. In Figure V - 2A, in each pair the sample that contains higher concentration of MWP was perceived significantly sweeter than the one containing less MWP with the exceptions of pairs 0-7/5-5 and 5-2.8/7-2. The increased sweetness of the samples containing MWP originated probably from the lactose, which is contained in the MWP at concentrations of 12 - 30% (dry basis) (Cheftel *et al.*, 1993). In Figure V - 2B, among all the pairs there was no significant difference in perceived thickness between the samples with comparable viscosity with the exception of 7-2/13-0 ( $p = 0.05$ ). This indicated that these samples with comparable viscosity were generally perceived same in thickness by the naive panel.

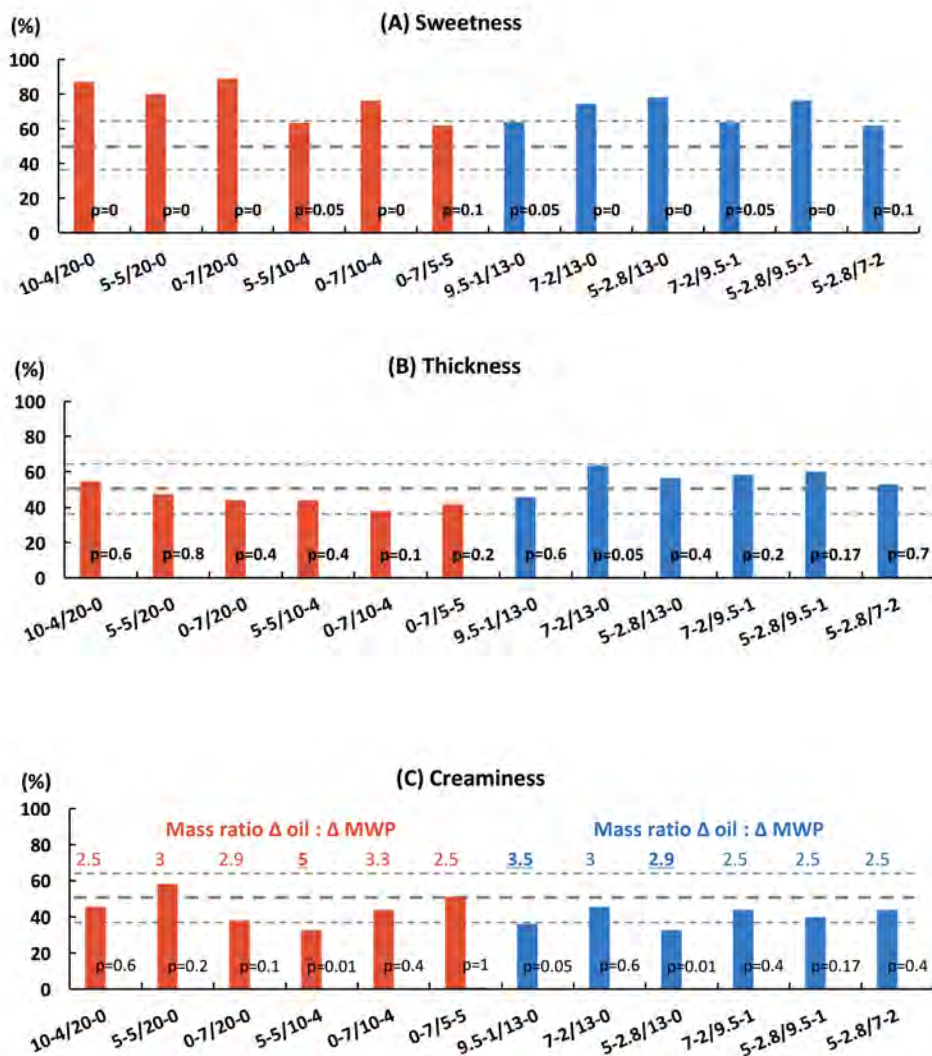


Figure V - 2 Paired-comparison between MWP-o/w emulsions with comparable viscosity for (A) Sweetness; (B) Thickness; (C) Creaminess. N=55 naive participants assessed all pairs. Sample codes and p-values are shown in all figures. Mass ratio  $\Delta$  oil :  $\Delta$  MWP (as calculated in Table V - 2) are shown in (C). Red bars represent samples with viscosity of 2.3 mPa·s, blue bars represent samples with viscosity of 1.7 mPa·s.

In Figure V - 2C, among all the pairs there was no significant difference in perceived creaminess between the samples except for pair 5-5/10-4 in the high viscosity group (2.3 mPa·s), and the pairs 9.5-1/13-0 and 5-2.8/13-0 in the low viscosity group (1.7 mPa·s). This demonstrated that MWP could replace oil in liquid emulsions to some extent while maintaining creaminess perception.

In Figure V - 2C, in the high viscosity group, the mass ratio  $\Delta\text{oil} : \Delta\text{MWP}$  of pair 5-5/10-4 is 5. This meant that in order to maintain the same viscosity, 1% (w/w) of MWP was added to replace 5% (w/w) of oil. However, this ratio was not sufficient to maintain creaminess perception ( $p = 0.01$ ). A mass ratio of at least 3.3 was needed to maintain thickness and creaminess perception ( $p > 0.05$ ) in the high viscosity group. In the low viscosity group, a mass ratio of at least 2.5 was needed to maintain thickness and creaminess perception ( $p > 0.05$ ). Mass ratio of higher than 2.9 was not sufficient to maintain creaminess perception ( $p \leq 0.05$ ), with the exception of pair 7-2/13-0, which had a mass ratio of three.

The results indicated that the mass ratio (needed to maintain thickness and creaminess perception) in the high viscosity group (3.3) was higher than in the low viscosity group (2.5). This indicates that MWP is able to replace more fat while maintaining creaminess in a higher viscosity matrix. Higher viscosity of the medium might be able to mask the coarseness of the particles (Sala *et al.*, 2015)2015, and coarseness is often reported to have negative effects on creaminess perception (de Wijk *et al.*, 2006c; Soukoulis *et al.*, 2010; Specter & Setser, 1994). This is confirmed by results of QDA in this study (5.3.1.4 & 5.3.2.1).

#### 5.3.1.4 Quantitative descriptive analysis (QDA) of MWP-o/w emulsions

Table V - 3 summarizes the sensory attributes and their definitions for MWP-o/w emulsions that were generated by the panel. Table V - 4 gives an overview of the mean intensity scores of all sensory attributes for MWP-o/w emulsions with standard deviations and homogenous grouping. Figure V - 3 shows the Principal Component Analysis (PCA) plot of attributes that are significantly different between all MWP-o/w emulsions.

**Table V - 3 List of attributes (with definitions) generated by the QDA panel for MWP-o/w emulsions.**

<b>MWP-o/w emulsions</b>		
<b>Attribute type</b>	<b>Attribute name</b>	<b>Description</b>
Taste	T-intensity	Total amount of flavor
	T-sweet	Basic flavor and artificial sweetener
	T-bitter	Bitter of the skin of walnuts
	T-milk	Milk, cooled milk after heating
	T-soap	Coconut soapy flavor
Mouthfeel	MF-thickness	From skimmed to whole milk
	MF-fatty	Oily, greasy, feel on the lips
	MF-creamy	Velvety, warm, soft, full
	MF-powdery	Chalk, lime, small particles in the liquid
	MF-dry coating	Dry film on teeth, tongue and / or cheeks
	MF-rough	Rough feel to the teeth and / or tongue, not smooth or slippery
Aftertaste	AT-metal	Egg eat with a silver spoon
	AT-bitter	Bitter of the skin of walnuts
	AT-milk	Milk, cooled milk after heating
Afterfeel	AF-fatty	Greasy coating on the tongue, lips or cheek inside
	AF-creamy	Velvety, warm, soft
	AF-rough	Dry, rough feel to the teeth and / or tongue, not smooth or slippery
	AF-powdery coating	Powdery film on teeth, tongue and / or cheeks, chalk, lime

**Table V - 4 Mean intensity scores  $\pm$  standard error for all sensory attributes for MWP-o/w emulsions. Equal letters indicate the homogenous groups ( $p < 0.05$ ).**

Attribute\Product	Average viscosity = 2.3 mPa·s				Average viscosity = 1.7 mPa·s			
	(20-0)	(10-4)	(5-5)	(0-7)	(13-0)	(9.5-1)	(7-2)	(5-2.8)
T_intensity	60.1 $\pm$ 3.3 a	44.1 $\pm$ 3.6 b	43.4 $\pm$ 3.3 b	65.6 $\pm$ 3.9 a	63.4 $\pm$ 3.9 a	46.9 $\pm$ 3.6 b	41.3 $\pm$ 3.6 b	41.9 $\pm$ 1.3 b
T_sweet	22.6 $\pm$ 2.4 bcd	30.6 $\pm$ 3.2 ab	29.0 $\pm$ 2.3 abc	37.0 $\pm$ 3.5 a	17.4 $\pm$ 1.4 d	20.5 $\pm$ 2.0 cd	21 $\pm$ 2.2 cd	21.7 $\pm$ 0.9 bcd
T_bitter	51.5 $\pm$ 3.9 ab	25.4 $\pm$ 2.2 de	23.1 $\pm$ 2.6 e	28.9 $\pm$ 3.5 cde	51.9 $\pm$ 3.9 a	40.1 $\pm$ 3.1 bc	35.3 $\pm$ 3.4 cd	28.1 $\pm$ 1.2 de
T_milk	26.6 $\pm$ 3.3 de	47.9 $\pm$ 3.9 abc	60.0 $\pm$ 3.8 a	52.1 $\pm$ 4.1 ab	23.6 $\pm$ 3.3 e	26.4 $\pm$ 2.5 de	38.2 $\pm$ 3.7 cd	42.6 $\pm$ 1.4 bc
T_soap	53.1 $\pm$ 4.7 a	22.5 $\pm$ 2.7 c	18.8 $\pm$ 2.4 c	32.2 $\pm$ 4.8 bc	52.7 $\pm$ 4.6 a	45.4 $\pm$ 3.8 ab	26.2 $\pm$ 3 c	25.9 $\pm$ 1.5 c
MF_thickness	58.3 $\pm$ 3.9 a	55.0 $\pm$ 3.9 a	40.5 $\pm$ 3.4 bc	35.3 $\pm$ 3.7 c	52.2 $\pm$ 4.7 ab	39.4 $\pm$ 3.5 bc	37.1 $\pm$ 3.6 c	36.7 $\pm$ 1.4 c
MF_fatty	53.3 $\pm$ 3.9 a	34.4 $\pm$ 3.5 b	23.4 $\pm$ 2.0 c	22.7 $\pm$ 2.3 c	49.3 $\pm$ 4.2 a	36.1 $\pm$ 3.2 b	26.5 $\pm$ 2.3 bc	25.9 $\pm$ 1.2 bc
MF_creamy	49.5 $\pm$ 4.4 a	44.1 $\pm$ 3.4 ab	32.0 $\pm$ 3.3 bcd	29.7 $\pm$ 3.6 cd	41.3 $\pm$ 4.4 abc	33.4 $\pm$ 3.2 bcd	27.5 $\pm$ 3.1 d	28.2 $\pm$ 1.3 d
MF_powdery	15.1 $\pm$ 1.9 e	30.5 $\pm$ 3.3 bc	35.8 $\pm$ 3.5 ab	45.8 $\pm$ 4.8 a	17.5 $\pm$ 2.2 de	22.8 $\pm$ 2.3 cde	26.9 $\pm$ 2.9 bcd	29.5 $\pm$ 1.2 bc
MF_dry_coating	17.9 $\pm$ 2.0 e	38.4 $\pm$ 3.3 bc	45.2 $\pm$ 3.8 ab	54.4 $\pm$ 4.4 a	22.4 $\pm$ 3.0 de	29.0 $\pm$ 2.7 cd	35.7 $\pm$ 3.1 cb	39.0 $\pm$ 1.3 cb
MF_rough	26.0 $\pm$ 3.1 d	45.2 $\pm$ 3.3 bc	52.4 $\pm$ 3.2 ab	60.1 $\pm$ 4.1 a	28.1 $\pm$ 2.8 d	34.4 $\pm$ 3.1 cd	45.1 $\pm$ 3.4 bc	47.8 $\pm$ 1.3 b
AT_metal	24.1 $\pm$ 2.6 bc	16.5 $\pm$ 1.5 d	14.5 $\pm$ 1.0 d	17.9 $\pm$ 1.7 cd	31.5 $\pm$ 3.7 a	25.7 $\pm$ 2.5 ab	20.6 $\pm$ 2.2 bcd	21.5 $\pm$ 0.8 bcd
AT_bitter	45.4 $\pm$ 3.7 a	21.1 $\pm$ 1.8 d	22.4 $\pm$ 2.8 d	25.9 $\pm$ 3.0 cd	43.5 $\pm$ 3.8 ab	34.8 $\pm$ 2.9 bc	27 $\pm$ 2.6 cd	26.2 $\pm$ 1.1 cd
AT_milk	22.1 $\pm$ 2.4 d	47.7 $\pm$ 3.7 ab	54.8 $\pm$ 3.5 a	50.3 $\pm$ 4.2 a	22.1 $\pm$ 2.4 d	25.4 $\pm$ 2.5 cd	33.7 $\pm$ 3.7 cd	36.8 $\pm$ 1.3 bc
AF_fatty	49.2 $\pm$ 3.8 a	32.0 $\pm$ 3.5 b	18.0 $\pm$ 1.7 c	18.7 $\pm$ 2.1 c	45.0 $\pm$ 3.9 a	31.8 $\pm$ 3.2 b	26.0 $\pm$ 2.7 bc	21.9 $\pm$ 1.2 bc
AF_creamy	40.2 $\pm$ 4.0 a	38.6 $\pm$ 3.8 a	23.8 $\pm$ 2.8 b	21.8 $\pm$ 2.9 b	35.0 $\pm$ 4.5 a	29.1 $\pm$ 3.2 ab	21.4 $\pm$ 2.5 b	21.5 $\pm$ 1.2 b
AF_rough	27.7 $\pm$ 2.9 e	43.5 $\pm$ 2.9 cd	57.5 $\pm$ 3.6 ab	66.1 $\pm$ 3.6 a	28.3 $\pm$ 3.0 e	38.5 $\pm$ 3.2 de	46.9 $\pm$ 3.1 bcd	51.1 $\pm$ 1.3 bc
AF_powdery_coating	17.3 $\pm$ 2.2 d	34.8 $\pm$ 3.2 bc	39.6 $\pm$ 3.6 b	52.2 $\pm$ 4.5 a	18.6 $\pm$ 1.9 d	26.4 $\pm$ 2.7 cd	30.4 $\pm$ 2.9 bc	36.4 $\pm$ 1.2 bc

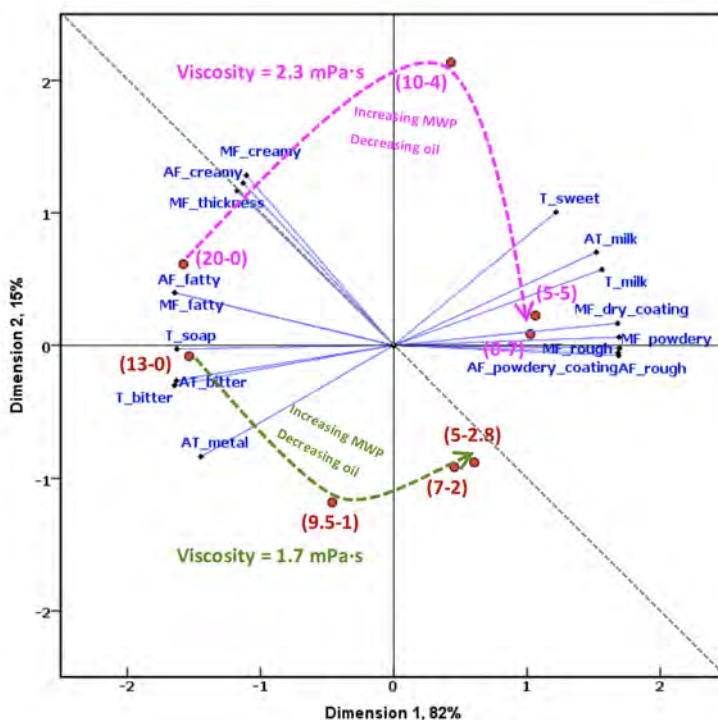


Figure V - 3 Principal Component Analysis (PCA) plot of attributes that are significantly different between samples obtained from QDA of MWP-o/w emulsions. Lines are to guide the eyes.

Significant differences were observed for all sensory attributes between MWP o/w emulsions (Table V - 4). In the following discussions, we focus on the mouthfeel and after-feel attributes. In Figure V - 3, the two principal components explain 97% of all the variation between the samples. PC1 accounts for most of the variance (82%) and distinguishes the samples varying from fatty/thickness/creamy to dry/rough. On the left side of PC1, MF(AF)\_creamy, MF\_thickness, and MF(AF)\_fatty are positively related to each other, and on the opposite side, MF\_dry coating, MF(AF)\_powdery, and MF(AF)\_rough are positively related to each other.

According to the definitions of the attributes, MF(AF)\_fatty was defined as oily/greasy coating on the oral surfaces. Sample 20-0 and 13-0 showed significant higher fatty intensity than other samples. With decreasing oil content and increasing MWP content, emulsions were perceived less fatty and more rough. This suggests that MF(AF)\_fatty might be related to the lubrication properties of the emulsion droplets. On one hand the small oil droplets



could easily roll on the oral surfaces thus providing lubrication, on the other hand some oil droplets might form film patches on oral surfaces due to droplet coalescence during oral processing, which provided the fatty coating perception.

The attributes MF\_dry coating, MF(AF)\_powdery, and MF(AF)\_rough were not only related to the decreasing oil content, but mainly from the presence of MWP particles. Due to the neutral pH of the samples, it is expected that these attributes are not related to the presence of whey proteins (Ye *et al.*, 2011). The particle size distribution of MWP in this study showed two peaks at around 0.4  $\mu\text{m}$  and 7.0  $\mu\text{m}$ . MWP particles that were larger than 50  $\mu\text{m}$  were observed from scanning electron microscopic analysis (Liu *et al.*, 2016). The reported size threshold for detection of particles in mouth is inconsistent among different literature. As discussed in the introduction, the detection of particles is affected by the size, concentration, shape, and hardness of the particles, as well as the properties of the matrix in which the particles are dispersed. For example, it was reported that the minimum size of particles in chocolate that could be detected by the palate is around 25  $\mu\text{m}$  (Minifie, 2012). Sharp-edged silica particles as small as 2  $\mu\text{m}$  already resulted in a roughness perception (Engelen *et al.*, 2005a). It was reported that in aqueous dispersion, particles larger than 3  $\mu\text{m}$  were sensed as powdery, chalky and gritty (Singer, 1996). It is assumed that a thicker matrix can mask the detection of particles in mouth (Sala *et al.*, 2015)2015. This might be the reason why literature has mentioned the roughness and dry coating perception of MWP scarcely. Mostly MWP are applied into much more viscous foods or food model systems than the liquid emulsions in the current study. Thus, the observed increase in roughness resulted from the large MWP particles with sizes above the detection threshold.

The attribute MF\_thickness was not correlated with measured viscosity. Possibly that the difference in viscosities between the two groups was too small ( $\Delta = 0.6 \text{ mPa}\cdot\text{s}$ ) that was very close to the reported “just noticeable difference” in oral thickness perception (using a Weber fraction  $K = 0.26$ ) (Camacho *et al.*, 2015).

Similar to our study, a “rough-fatty/creamy” dimension was observed in two studies regarding vanilla custards (de Wijk *et al.*, 2006b; de Wijk *et al.*, 2003). In these two reported studies, this dimension was primarily driven by fat content. The “creamy” perception was at the same direction as “fatty” perception. In our study, PC2 distinguished attributes MF(AF)\_creamy from MF(AF)\_fatty. This suggested that although creamy perception was highly related to fat content, other factors also contributed to creamy perception. Several points regarding the contribution of MWP particles, oil droplets, and viscosity, to the creamy perception are discussed below.

First, regarding the contribution of MWP particles, creaminess perception of emulsions 5-5, 0-7, 9.5-1, 7-2, and 5-2.8 were not significantly different. These emulsions had oil contents ranging from 9.5% to 0%. Apparently, in this range of oil content the MWP particles could replace oil droplets successfully and provided similar creamy mouthfeel and afterfeel. When reducing oil content from 20% (20-0) to 10% (10-4), addition of 4% MWP (10-4) could maintain creaminess at a similar level as the 20-0 emulsion. These results can be attributed to the lubrication properties of MWP particles due to their free rolling and spherical shape (ball bearing) (Liu *et al.*, 2016). In addition, these results give an indication that the presence of small particles was important for creamy perception. The size and shape of the particle is of great importance as we have discussed before about the rough and powdery perception. Particles that were smaller than the detection threshold could enhance creaminess (Kilcast *et al.*, 2002). The existence of small undetectable particles was important for a so-called “substantialness” impression, which was synonymous with “body” and “richness”. Absence of the small particles might give the “watery” perception (Singer, 1996). In contrast, big particles that imparted rough or powdery could suppress creaminess (de Wijk *et al.*, 2006a; Singer, 1996; Wood, 1974).

Second, regarding the contribution of oil, from the PCA graph we see that emulsions 20-0, 10-4, and 13-0 were in a group that was perpendicular to the dimension of creamy. However, all other emulsions, forming a group that was also perpendicular to the dimension of creaminess, were located opposite of emulsion 20-0. In general, emulsions containing high oil content (e.g. > 10%) were perceived with higher creaminess perception. Emulsions 7-2 and 5-2.8 were both perceived significantly less creamy than emulsion 10-4, although emulsion 10-4 has more MWP that can suppress creamy mouthfeel. In addition, although increasing the concentration of MWP resulted in better lubrication (Figure V - 1), creaminess did not follow this trend. These results indicate that the roles that oil plays in providing creaminess cannot be fully replaced by MWP.

Third, regarding the contribution of viscosity, the attribute MF(AF)\_creamy as well as MF\_thickness were not always correlated to the measured viscosity. This suggested that in our study, where the viscosity range was very limited (from 1.7 to 2.3 mPa-s), viscosity had only limited effect on the perceived creaminess. It was reported that even at a wider range of viscosities (from 2.6 to 26 mPa-s), there was a lack of perceived difference in creaminess of liquid dairy products that could be related to different viscosities (Richardson-Harman *et al.*, 2000). This was attributed to a larger sensory effect of fat content on perceived creaminess that might have masked the differences in viscosities.

To summarize, based on the above discussions regarding perception of fattiness, roughness, and creaminess, we conclude that oil contributes to creaminess in two aspects. First, oil can reduce friction by ball bearing lubrication (in the case of stable oil droplets) and film lubrication (in the case of coalesced droplets that form film patches) (Liu *et al.*, 2016). Lubrication contributes positively to perception of creaminess. Second, the formation of oil film patches on oral surfaces contributes to perception of fattiness, which enhances the perception of creaminess. With respect to MWP particles, we conclude that MWP influences creaminess in four aspects. First, MWP reduce friction by ball bearing lubrication, which contributes positively to creaminess. Second, the presence of small MWP particles (as well as oil droplets) provides “body” and “richness” which contributes positively to creaminess. Third, MWP is not able to form film patches thus not able to contribute to perception of fattiness. In this point of view, MWP contributes neutrally to creaminess. Fourth, MWP with size larger than detection threshold provides rough and dry perception, which contributes negatively to creaminess. In the end, in order to maintain optimal creaminess by replacing oil with MWP, it is important to have a balanced ratio of oil and MWP to maximize the positive contributions of both oil and MWP and minimize the negative contributions of MWP.

### 5.3.2 Gel samples: MWP-emulsion-filled gels

#### 5.3.2.1 Large deformation properties

Figure V - 4A shows the Young's modulus of MWP-emulsion-filled gels at different MWP and oil concentration. The oil droplets were stabilized with 1%WPI, therefore the droplets were regarded to be bound to the gelatin matrix (Liu *et al.*, 2015). For gels without MWP, with increasing oil concentration from 5 to 20%, Young's modulus increased significantly. This confirmed that oil droplets were bound to the matrix. For gels containing 5 and 15% oil, increasing MWP from 0 to 1% did not significantly increase the modulus. Further increasing MWP from 1 to 4% significantly increased the modulus. This was in accordance with our previously reported data and confirmed that MWP particles were also, like the oil droplets, bound to the gelatin matrix (Liu *et al.*, 2016).

Figure V - 4B and C show the fracture stress and strain of the above-mentioned gels. Neither MWP nor oil droplets showed an obvious trend in influencing the fracture stress of the gels. The fracture strain of emulsion-filled gels without MWP slightly decreased with increasing oil content. No significant influence of MWP was found on the fracture strain of these gels. These results are in agreement with reported data (Liu *et al.*, 2015; Liu *et al.*, 2016). According to Figure V - 4D, the recoverable energy of the gels was not significantly affected

by the inclusion of either MWP particles or oil droplets, since both MWP and oil droplets were bound to the gelatin matrix (Liu *et al.*, 2015; Liu *et al.*, 2016; Sala *et al.*, 2009b).

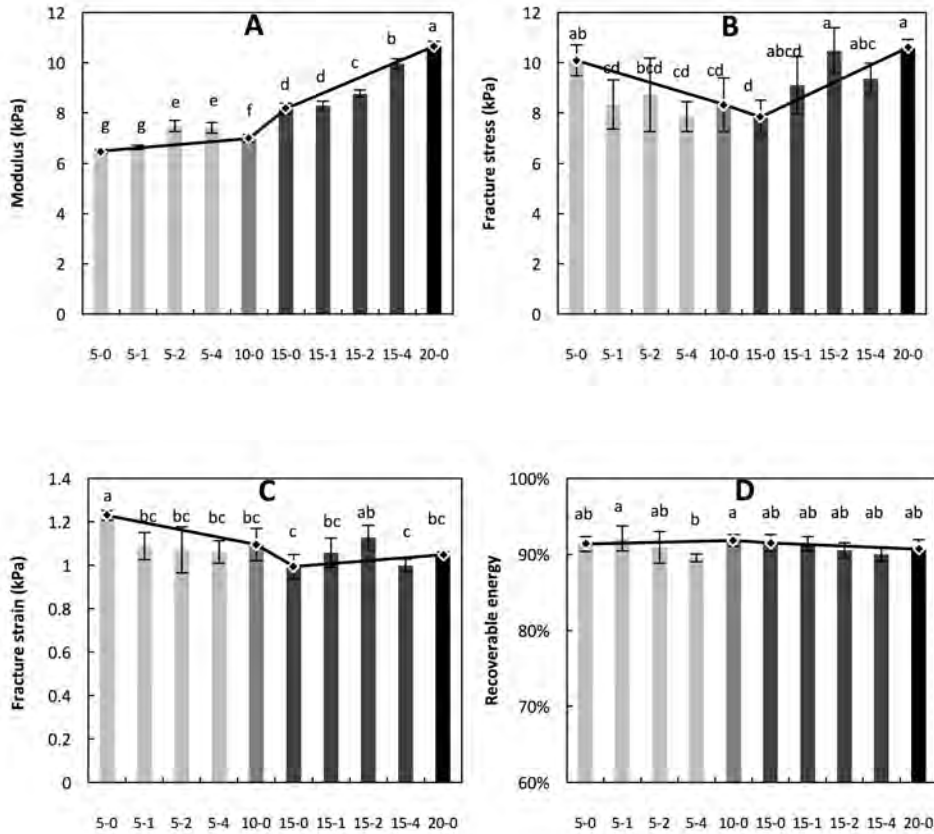


Figure V - 4 Large deformation properties of MWP-emulsion-filled gels at different MWP and oil concentration. A) Young’s modulus; B) Fracture stress; C) Fracture strain; D) Recoverable energy. Blue diamond symbols and blue lines indicate the samples without MWP. Error bars represent the standard deviation of 6 to 8 measurements. Equal letters indicate the homogenous groups ( $p < 0.05$ ). Bars with increasing darkness indicate increasing oil concentration from 5 to 20%.

### 5.3.2.2 Tribology

Figure V - 5A shows the friction coefficient of emulsion-filled gels containing different amounts of oil. For gels that did not contain MWP, the friction coefficient decreased with increasing oil concentration from 0 to 20% and decreased with increasing sliding speed from 10 to 80mm/s. Figure V - 5B shows that for gels containing 5% oil, increasing MWP from 0

to 4% induced a reduction in friction coefficient. Figure V - 5C shows similar effect of MWP on gels containing 15% of oil. These results were also in line with previously reported data (Liu *et al.*, 2015; Liu *et al.*, 2016). These results confirmed that both oil droplets and MWP particles could improve the lubrication property of gelatin gels used in this study.

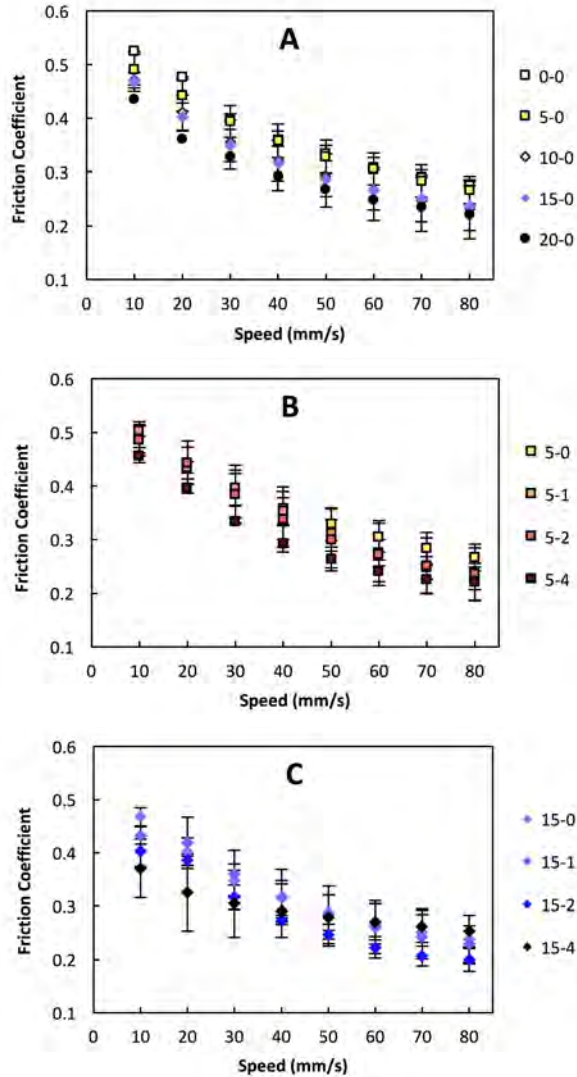


Figure V - 5 Friction coefficients of MWP-emulsion-filled gels. Error bars represent the standard deviation of triplicate measurements.

5.3.2.3 Quantitative descriptive analysis (QDA) of MWP-emulsion-filled gelatin gels

Table V - 5 summarizes the sensory attributes as well as their definitions for MWP-emulsion-filled gelatin gels generated by the trained panel. Table V - 6 gives an overview of the mean scores of QDA sensory attributes for MWP-emulsion-filled gels with standard deviations and homogenous grouping. Figure V - 6 shows the PCA plot of attributes that are significantly different among all MWP-emulsion-filled gels.

**Table V - 5 List of attributes generated during the training of QDA of MWP-emulsion-filled gel samples and definitions of these attributes**

<b>MWP-emulsion-filled gels</b>		
<b>Attribute type</b>	<b>Attribute name</b>	<b>Description</b>
Taste	T-intensity	total amount of taste
	T-oil	sunflower oil, seed coat of nuts
	T-milk	milk taste
	T-metal	like eating an egg with silver spoon, ionizing
	T-soap	alkaline, basic taste
	T-nutty	taste of nuts
Mouthfeel	MF-slippery	slips away easily, smooth
	MF-firm	force is required to press
	MF-elastic	resilient , like a rubber band
	MF-spreadable	upon pushing with the tongue, it becomes a kind of paste
	MF-crumby	falls apart into pieces
	MF-powdery	as cornstarch, flour
	MF-melting	dissolves in your mouth, structure disappears
	MF-fatty	oily, greasy coating on the tongue, lips and / or inner cheeks
	MF-creamy	full, soft, velvety mouthfeel
Aftertaste	AT-milk	aftertaste of milk
	AT-oil	sunflower oil, seed coat of nuts
After-feel	AF-fatty	oily, greasy coating on the tongue, lips and / or inner cheeks
	AF-creamy	full, soft, velvety mouthfeel
	AF-sticky	sticky on the lips
	AF-dry	dry, powdery, lack of fluid
	AF-rough	unripe banana, the feeling of sledging without snow

**Table V - 6 Mean scores ± standard error of QDA sensory attributes for MWP-emulsion-filled gels. Equal letters indicate the homogenous groups (p < 0.05).**

Attribute\Product	(5-0)	(5-1)	(5-2)	(5-4)	(10-0)	(15-0)	(15-1)	(15-2)	(15-4)	(20-0)
T_intensity	47 ± 3.3 d	50.4 ± 2.9 cd	51.9 ± 3.0 cd	59.8 ± 3.0 abc	55.9 ± 2.6 bcd	56.9 ± 2.7 bcd	62.6 ± 2.8 ab	60.1 ± 2.2 abc	67.8 ± 2.2 a	62.7 ± 2.7 ab
T_oil	41.2 ± 3.2 bc	43.5 ± 2.9 bc	45.2 ± 3.2 abc	40.0 ± 3.2 c	47.8 ± 2.9 abc	52.0 ± 3.4 abc	56.8 ± 3.5 a	52.0 ± 2.9 abc	52.7 ± 3.6 ab	57.2 ± 3.1 a
T_milk	22 ± 2.1 d	29.4 ± 2.9 cd	37.6 ± 3.6 bc	54.9 ± 3.9 a	29.3 ± 3.1 cd	29.4 ± 2.7 cd	36.2 ± 3.3 bc	45.5 ± 4.2 ab	56.6 ± 4.5 a	32.7 ± 3.6 bcd
T_metal	32.9 ± 4.0 a	26.9 ± 3.8 abc	20.8 ± 2.0 bcd	20.6 ± 2.1 bcd	27.5 ± 3.5 ab	27.4 ± 3.3 abc	24.7 ± 3.3 abcd	17.2 ± 1.6 cd	16.1 ± 1.7 d	26.9 ± 3.5 abc
T_soap	27.9 ± 3.8 ab	24.9 ± 3.1 abc	21 ± 2.3 abc	18.3 ± 1.8 bc	27.2 ± 3.5 abc	28.7 ± 3.4 a	26 ± 3.5 abc	23 ± 2.7 abc	17.8 ± 2.0 c	27.9 ± 2.9 ab
T_nutty	29.5 ± 3.1 d	30 ± 2.6 d	34.3 ± 3.3 bcd	32.6 ± 3.5 cd	34.6 ± 2.8 abcd	37.4 ± 3.8 abcd	44.4 ± 4.2 ab	41 ± 3.9 abc	41.7 ± 3.6 abc	44.8 ± 3.7 a
MF_slippery	68.1 ± 3.0 ab	70.1 ± 3.0 a	70.6 ± 3.1 a	64.1 ± 2.3 abc	66.3 ± 2.5 ab	59.2 ± 3.5 abc	64.6 ± 2.9 abc	63.1 ± 3.2 abc	57.5 ± 3.9 bc	52.8 ± 3.5 c
MF_firm	43.4 ± 2.7 c	46.2 ± 3.4 bc	45.0 ± 3.0 c	54.2 ± 2.6 abc	46 ± 2.9 bc	49.7 ± 2.7 bc	53.4 ± 3.2 abc	56.9 ± 3.0 ab	63.3 ± 3.0 a	49.3 ± 3.2 bc
MF_elastic	40.5 ± 3.5 ab	47.3 ± 3.6 b	41.1 ± 3.3 b	50.8 ± 3.5 ab	41.7 ± 2.9 ab	41.0 ± 3.0 ab	42.8 ± 3.3 ab	44.9 ± 2.5 ab	47.8 ± 3.6 a	43.7 ± 3.4 a
MF_spreadable	52.7 ± 3.8 ab	50.1 ± 4.0 b	48.7 ± 3.6 b	55.8 ± 3.2 ab	57.5 ± 3.5 ab	57.9 ± 3.6 ab	60.6 ± 3.2 ab	56.7 ± 3.1 ab	63.3 ± 3.4 a	64.9 ± 2.8 a
MF_crumbly	46.5 ± 3.4 ab	53.1 ± 3.4 b	52.8 ± 3.1 b	48.6 ± 3.6 ab	50.8 ± 3.1 ab	47.0 ± 3.6 ab	46.0 ± 3.3 ab	49.6 ± 3.8 ab	48.1 ± 3.8 ab	47.5 ± 3.5 ab
MF_powdery	23.7 ± 3.3 ab	21.8 ± 2.5 ab	24.5 ± 2.9 ab	29.8 ± 3.4 ab	23.1 ± 2.5 ab	26.5 ± 3.1 ab	27.0 ± 3.0 ab	31.6 ± 3.7 ab	29.7 ± 3.1 ab	29.3 ± 3.9 ab
MF_melting	64.3 ± 3.2 ab	63.4 ± 3.4 ab	60.0 ± 3.3 ab	58.7 ± 2.8 ab	61.5 ± 3.2 ab	63.4 ± 3.2 ab	63.0 ± 2.3 ab	59.3 ± 3.4 ab	53.6 ± 2.8 ab	59.1 ± 3.1 ab
MF_fatty	40.8 ± 3.8 bc	41.3 ± 3.4 bc	39.9 ± 3.2 c	47.5 ± 3.5 abc	47.9 ± 2.9 abc	54.1 ± 3.0 ab	54.2 ± 3.8 ab	53.7 ± 3.2 ab	56.4 ± 3.2 a	55.2 ± 3.5 a
MF_creamy	31.9 ± 3.2 e	36.8 ± 3.0 de	42.0 ± 3.5 cde	50.5 ± 3.3 bc	50.3 ± 3.3 bc	48.0 ± 3.4 bcd	54.6 ± 3.5 abc	57.1 ± 3.6 ab	64.9 ± 2.9 a	56.2 ± 3.5 ab
AT_milk	21.1 ± 2.3 e	32.5 ± 3.6 cde	36.7 ± 3.8 cd	55.4 ± 4.2 ab	29.7 ± 3.1 de	30.0 ± 3.2 de	38.8 ± 3.9 cd	43.0 ± 4.0 bc	56.1 ± 4.7 a	34.1 ± 3.8 cd
AT_oil	35.5 ± 3.3 bc	39.6 ± 3.1 abc	36.1 ± 3.3 bc	33.4 ± 3.7 c	43.1 ± 3.1 abc	46.0 ± 3.3 ab	49.0 ± 3.2 a	44.4 ± 3.0 abc	42.2 ± 3.3 abc	45.2 ± 3.3 abc

Attribute\Product	(5-0)	(5-1)	(5-2)	(5-4)	(10-0)	(15-0)	(15-1)	(15-2)	(15-4)	(20-0)
AF_fatty	33.4 ± 3.4 bcd	29.5 ± 2.4 cd	29.8 ± 2.6 cd	27.9 ± 2.7 d	37.1 ± 3.0 abcd	42.2 ± 2.9 ab	44.3 ± 3.3 ab	40.2 ± 2.9 abc	47.8 ± 3.8 a	48.3 ± 3.2 a
AF_creamy	25.6 ± 2.6 d	23.7 ± 2.4 d	29.3 ± 2.8 cd	39.2 ± 3.8 bc	28.9 ± 2.9 cd	37.9 ± 3.4 bc	41.9 ± 3.9 b	45.1 ± 3.9 b	57.6 ± 3.8 a	44.8 ± 4.0 b
AF_sticky	43.8 ± 4.4	43.7 ± 4.0	47.4 ± 4.1	52.9 ± 4.2	50.8 ± 4.2	50.0 ± 4.2	50.6 ± 4.4	47.9 ± 3.9	49.6 ± 3.5	48.0 ± 3.8
AF_dry	34.4 ± 3.7	38.8 ± 3.2	37.3 ± 3.2	43.6 ± 4.0	35.8 ± 3.0	33.3 ± 2.7	33.4 ± 2.8	38.8 ± 3.2	36.3 ± 3.2	33.7 ± 2.7
AF_rough	35.7 ± 3.4	38.4 ± 3.0	37.7 ± 2.8	42.7 ± 3.5	35.7 ± 2.7	35.3 ± 3.0	35.4 ± 2.9	37.3 ± 2.6	33 ± 3.1	29.3 ± 2.7

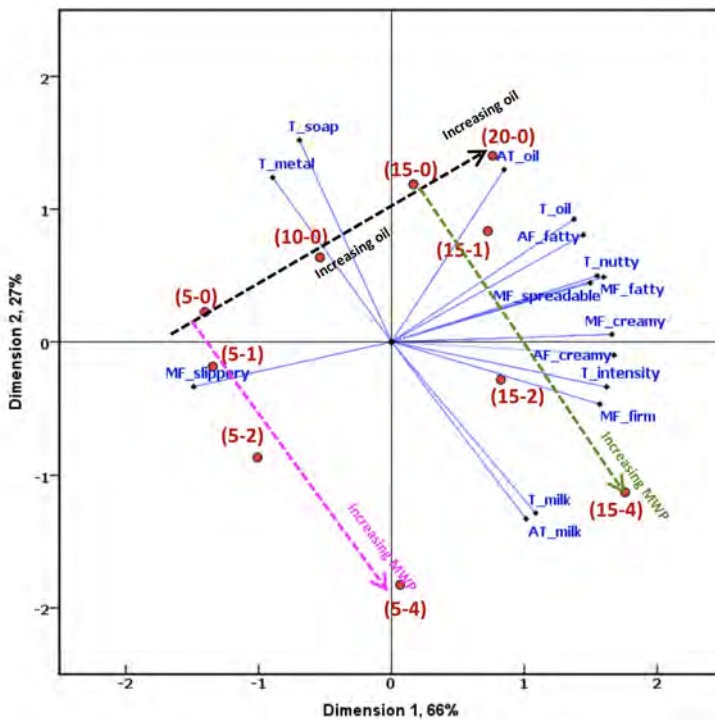


Figure V - 6 Principal Component Analysis (PCA) plot of attributes that are significantly different between MWP-emulsion-filled gels obtained from QDA. Lines in the plot are for guiding the eyes.

MWP-emulsion-filled gels were perceived not significantly different in seven mouthfeel and after-feel attributes: MF\_elastic, MF\_crumbly, MF\_powdery, MF\_melting, AF\_sticky, AF\_dry, and AF\_rough. Mouthfeel attributes MF\_elastic and MF\_crumbly were related to



the recoverable energy and fracture strain of the gels, which were not significantly different between most of the gels (Figure V - 4C and D). Attributes MF\_melting and AF\_sticky were probably related to the properties of the gelatin matrix, which was the same (4% gelatin) for all gels. Different from the results of the liquid MWP-o/w emulsions, MWP-emulsion filled gelatin gels differing in MWP content were perceived not significantly different in MF\_powdery, AF\_dry, and AF\_rough. Since the gelatin matrix in the gels was much thicker than the liquid emulsions, the detection of particles in mouth was suppressed (Sala *et al.*, 2015)2015. In addition, MF\_firm was related to the Young's modulus of the gels (Finney, 2012; Liu *et al.*, 2015), which were both increased with increasing MWP and oil content (Figure V - 4A).

Figure V - 6 shows that the first two principal components explain 93% of all the variation between the samples. MF\_slippery was located as a separated attribute on the left side of PC1, while most of other attributes were clustered on the right side of PC1. PC2 clearly distinguished T\_soap, T\_metal, and T(AT)\_oil from T(AT)\_milk. PC2 also distinguished MF(AF)\_fatty from MF(AF)\_creamy.

Dimension of MF(AF)\_fatty was parallel with the direction of gels with increasing oil content, while perpendicular to the direction of gels with increasing MWP content. This indicated that fatty perception is unique for oil-containing gels. This was in line with our earlier discussion for the liquids (section 5.3.1.4). Similar to the PCA graph of the emulsions (Figure V - 3), direction of attribute MF(AF)\_creamy deviated from MF(AF)\_fatty. Attribute MF(AF)\_creamy increased significantly with both increasing oil content (in absence of MWP) and MWP content (constant oil content). In reference to the preceding discussions regarding the liquid emulsions, we cannot deny that the lubrication functions of both oil droplets and MWP particles contributed to creaminess. It seemed that the contribution of MWP particles to creaminess in gels was more evident than in emulsions. One might expect the opposite since the ball-bearing lubrication of MWP particles in gels was less effective than in liquids (Liu *et al.*, 2016). However, creaminess of MWP in gels might no longer be suppressed as in liquids due to the absence of powdery and rough mouthfeel in gel systems. In the end, the net effect of MWP particles in gel systems would be enhancing the creamy perception.

With increasing MWP content at constant oil content, the attribute T(AT)\_milk increased, while attributes T\_soap and T\_metal decreased. This indicated that the attributes T\_soap and T\_metal were related to oil content and were suppressed by the attributes T\_milk and AT\_milk. Although the rough-related attributes caused by MWP particles were not perceived or distinguished among the gel samples, the taste attributes were still perceived and could

be distinguished among different samples. This indicated that the thicker gel matrix was only able to mask the perception of big particles but not small taste or flavor molecules due to their faster diffusion rate.

## 5.4 Conclusions

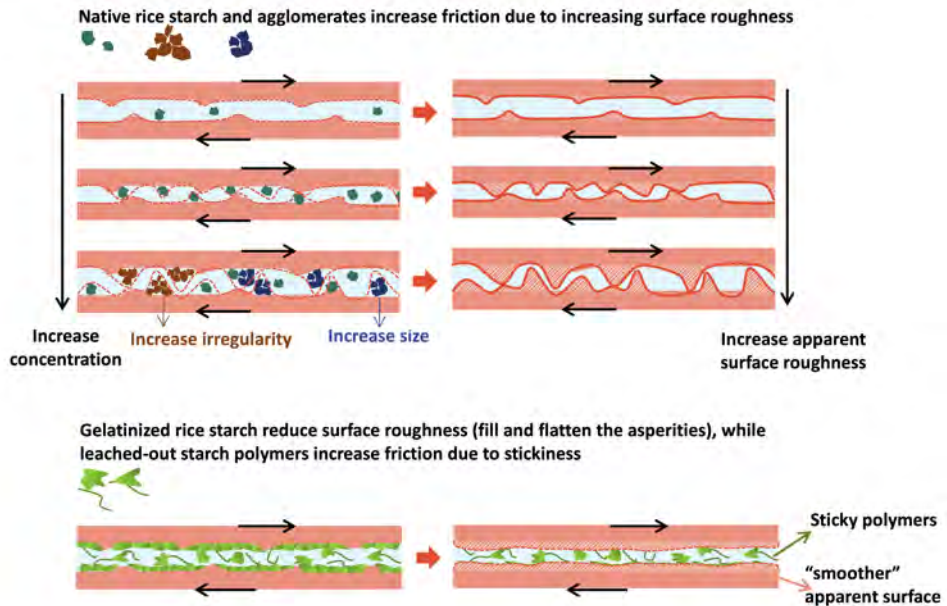
We investigated the sensory properties of MWP particles in relation to their rheological and tribological properties in liquid emulsions and semi-solid emulsion-filled gels. Consistent with our previous findings, MWP showed good lubrication properties in both liquid and semi-solid systems as measured by tribology. MWP could be used as a texture modifier due to its influence on viscosity of emulsions and on texture properties of gels.

Sensory detection of the particles is related to the size of the particle as well as the properties of the surrounding matrix. MWP particles that were smaller than the detection threshold contributed to perception of creaminess due to its lubrication property, but did not contribute to perception of fattiness in contrast to oil droplets. MWP particles that were bigger than the size corresponding to the detection threshold might have contributed to the rough and powdery perception, and thus suppressed perception of creaminess. Therefore, MWP in liquid emulsions were generally perceived as rough but not creamy. In addition, perception of thickness of MWP-o/w emulsions was not only dependent on the viscosity of the studied system.

Due to a combined effect of both oil droplets and MWP particles, creamy mouthfeel and after-feel of the MWP-emulsion filled gelatin gels were enhanced. Although gel matrix restrained the lubrication function of MWP particles, it also masked the rough perception of big MWP particles (compared to in liquid emulsions). In the end, MWP in gels resulted in an overall positive effect on the creamy perception. Thus, it is of great importance to consider the effect of food matrix when apply particle fat replacers in foods.

# Chapter 6

## Tribological properties of rice starch in liquid and semi-solid food model systems



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## Abstract

This study investigated the tribological and rheological properties of liquid and semi-solid food model systems containing micro-granular rice starch. Native (uncooked) and gelatinized rice starch dispersions, o/w emulsions and emulsion-filled gelatin gels were studied as food model systems. Native rice starch particles behaved as active fillers and increased the gel modulus with increasing concentration. At low concentration, soft gelatinized rice starch particles decreased gel modulus. At high concentration, gelatinized rice starch increased the gel modulus due to dense packing of particles. Native and gelatinized rice starch increased friction coefficients with increasing concentration due to different mechanisms. Native rice starch particles increase friction probably due to their irregular shape and particle agglomerations (at high concentration) that indirectly increase surface roughness and asperity contacts. Gelatinized rice starch particles increase friction probably due to the stickiness of leached-out starch polymers. The presence of oil droplets in the case of rice starch-o/w emulsions could reduce the friction caused by stickiness of gelatinized rice starch. The tribological behaviors of rice starch-emulsion-filled gels are more complex than liquids due to the bulk properties and breakdown properties of gel matrix. We conclude that morphology and surface properties of the starch particles, bulk and breakdown properties of matrices are the main factors that determining the tribological properties of food model systems containing rice starch particles. Our results may give an indication that the fat-mimicking functionality of rice starch is probably not due to their contribution to lubrication, but due to other factors, such as contributions to thickness and melting.

## **6.1 Introduction**

In recent decades, the growing trend for consuming low fat or fat-free foods has led to the development of fat replacers with low caloric values. Fat plays multiple roles in food products and gives foods unique functional and sensory characteristics (Akhtar *et al.*, 2006; Chung *et al.*, 2013). One of the biggest challenges in fat reduction is to maintain the mechanical properties of foods and at the same time retain the appreciated sensory attributes that fat provides. Among all the relevant sensory attributes, creaminess is often the most appreciated yet the most difficult to maintain. Creaminess is a complex sensorial characteristic that is related to multiple food properties (Akhtar *et al.*, 2006). It has been suggested that creaminess is a combination of several other sensory attributes including (yet not limiting to) thickness, smoothness, slipperiness, and melting (Alting *et al.*, 2009; Kokini, 1987). Thickness and melting are primarily related to the viscosity of the food products, while smoothness and slipperiness are related to lubrication properties (Kokini, 1987; Kokini *et al.*, 1983).

Proteins and carbohydrates are candidates for fat replacers since they have lower energy densities than fat. Protein-based fat replacers are often proteins or protein-polysaccharide complexes in a microparticulated form (Roller *et al.*, 1996; Setser *et al.*, 1992). The size range of these microparticulated ingredients is often comparable with homogenized fat globules (a few microns) in many types of processed foods. It is postulated that microparticulated proteins in water-continuous food systems can be viewed to some extent as physically similar to an o/w emulsion (Roller *et al.*, 1996). On one hand, due to their micrometer size, these particles are assumed to be not detectable by tongue (de Wijk *et al.*, 2005; Engelen *et al.*, 2005b; Tyle, 1993). On the other hand, the spheroidal shape of the particles provides a lubrication effect due to a ball-bearing mechanism (Liu *et al.*, 2016), where lubrication is important for the perception of creamy and smooth mouthfeel.

Starch is one of most commonly used carbohydrate-based fat mimetics (Ognean *et al.*, 2006). In general, starch-based fat replacers were able to retain the texture of food by thickening, gelling and stabilizing substantial quantities of water (Malinski *et al.*, 2003). Starch granules, as well as the released starch polymers (and the hydrolyzed molecules) during food processing may act as hydrophilic colloids that increase the viscosity of the continuous phase (Eliasson, 2006). From this perspective, starch-based fat mimetics can replace fat by maintaining the mechanical properties and some sensory attributes, such as thickness. In addition, starch-based ingredients are known to be susceptible to hydrolysis during mastication by amylase present in saliva. Therefore, starch-based fat replacers

also contribute to the perception of melting. Some starches, such as enzymatic treated starch, display thermo-reversible behavior that can improve creaminess due to physical melting (Alting *et al.*, 2009). From this point of view, starch-based fat replacers can provide creaminess by mimicking the melting characteristics of fats.

Starch-based fat replacers mostly originate from corn, potato, tapioca, and wheat starches that have relatively large granule size (10 - 100  $\mu\text{m}$ ). However, it is often reported that these starches were not able to fully mimic creaminess of full fat foods and were often accompanied with undesirable perceptions, such as grittiness and graininess (Bakal *et al.*, 1992; de Wijk *et al.*, 2006b). The perception of these attributes results from the friction between the food particles and oral mucosa (de Wijk *et al.*, 2005). Knowing this, to achieve optimal mimicry of creaminess requires that the starches also fulfill the lubrication function.

Following the concept of microparticulated proteins, spheroidal particles with a few micrometer size composed of starch or other carbohydrates might also have the potential to provide lubrication in food products (Bakal *et al.*, 1992; N.S. Singer *et al.*, 1990). Microparticulated starch from modified large granular starch (Setser *et al.*, 1992) and native or modified micro-granular starch display particle sizes of a few micrometers. A wide range of micro-granular starches is available in nature, such as parsnip (1 - 6  $\mu\text{m}$ ), rice (2 - 8  $\mu\text{m}$ ), amaranth (1 - 2  $\mu\text{m}$ ), cow cockle (0.3 - 1.5  $\mu\text{m}$ ), quinoa starches (0.5 - 3  $\mu\text{m}$ ), and fine fractions of wheat starches (2 - 10  $\mu\text{m}$ ) (Jane *et al.*, 1994; Lindeboom *et al.*, 2004; Wani *et al.*, 2012). These micro-granular starches are not all perfectly spherical but polyhedral. Maliniski *et al.* (2003) reported that small wheat starch granules that are isolated from whole flour and separated from larger granules, as well as amaranth starch granules (0.5 - 1.2  $\mu\text{m}$ , polyhedral) could achieve up to 50% fat replacements in frozen desserts. Rice starches, either in raw or gelatinized form, were claimed to be able to serve as natural fat replacers in ice cream (Chigurupati *et al.*, 1992; Mason *et al.*, 2009), dressing (Bakal *et al.*, 1992), and sausages (Setser *et al.*, 1992). The small micro-granular starches could provide a smooth mouthfeel with sufficient body and creaminess (Moldenhauer *et al.*, 1998). The “fat-like” mouthfeel that small micro-granular starches provide is often attributed to their small particle size (Joly *et al.*, 2009) in a similar way as the microparticulated protein does. It has been suggested that these small starch granules in frozen dessert might lubricate ice crystals and amplify creaminess (Malinski *et al.*, 2003).

In summary, micro-granular starches might have the potential to combine the benefits of starch itself (which in general contributes to increasing viscosity and melting), with those of a microparticulated protein that improves lubrication. However, research on the

lubrication properties of micro-granular starches is scarce. Zinoviadou et al (2008) reported the tribological properties of several neutral polysaccharide solutions including gelatinized cross-linked tapioca starches (with average particle size about 50  $\mu\text{m}$ ), which are much larger than micro-granular starches that we are interested in. Even when looking at other types of micro-particles in foods, only a few studies on their tribological properties were reported, such as kappa carrageenan particles (Garrec *et al.*, 2013), whey protein aggregates (Chojnicka-Paszun *et al.*, 2014b; Chojnicka *et al.*, 2008), microcrystalline cellulose particles (Chojnicka-Paszun *et al.*, 2014a), and chocolate particles (Lee *et al.*, 2004; Luengo *et al.*, 1997). In general, limited information can be found regarding lubrication properties of particle-containing foods.

The aim of this study was to investigate the tribological properties of micro-granular native and gelatinized rice starch in liquid o/w emulsions and semi-solid emulsion-filled gels. This study contributes to the discussion on the possible mechanisms underlying the fat mimetic properties of micro-granular particles. We selected rice starch due to its small particle size and its wide availability compared with micro-granular starches from other botanical sources. In this paper, native rice starch refers to native rice starch that is uncooked, and gelatinized rice starch refers to native rice starch that has been gelatinized. Gelatin was chosen as the matrix for the semi-solid gels.

## **6.2 Materials & methods**

### **6.2.1 Materials**

Native rice starch (Remy DR, 17% amylose) was provided by Beneo (Belgium). Sunflower oil was obtained from a local supermarket (the Netherlands). Porcine skin gelatin (bloom value 240–260) was supplied by Rousselot (Gent, Belgium). Powdered whey protein isolate (WPI, Bipro™) was obtained from Davisco International Inc. (La Sueur, MN, USA). All materials were used without further purification. All samples were prepared with reverse osmosis water.

### **6.2.2 Sample preparations**

#### **6.2.2.1 Rice starch dispersions**

A stock dispersion of native rice starch was prepared by dispersing 10% (w/w) native rice

starch in water under gentle stirring for at least 2 h at room temperature. A stock dispersion of gelatinized rice starch (10%, w/w) was prepared by heating the stock dispersion of native rice starch in a waterbath at 68.5 °C for 20min. The stock native and gelatinized rice starch dispersions were then diluted with water to lower concentrations (Table VI - 1). All the starch dispersions were freshly prepared on the day of measurements. To minimize sedimentation, all samples were stirred until the moment of further analysis.

**Table VI - 1 Overview of the composition of all samples**

Native rice starch % (w/w)	Gelatinized rice starch % (w/w)	Oil % (w/w)	Gelatin % (w/w)
Rice starch dispersions			
0.5; 1; 2; 5; 8 <sup>a</sup> ; 10		-	-
	0.5; 1; 2; 5; 8 <sup>a</sup> ; 10	-	-
Rice starch-o/w emulsions			
0; 1; 2; 4; 6;		5	-
0; 1; 2; 4; 6;		20	-
	0; 1; 2; 4; 6;	5	-
	0; 1; 2; 4; 6 <sup>b</sup> ;	20	-
Rice starch-emulsion-filled gels			
0; 1; 2; 4;		5	4
0; 1; 2; 4;		20	4
	0; 1; 2; 4;	5	4
	0; 1; 2; 4;	20	4

<sup>a</sup> Viscosity of these samples were not measured; <sup>b</sup> Viscosity and friction data of this sample was not available due to sample defects;

#### 6.2.2.2 Rice starch-o/w emulsions

A stock o/w emulsion was prepared by mixing sunflower oil (40%, w/w) with aqueous phase (60%, w/w). The aqueous phase contained 1% (w/w) WPI as emulsifier. This oil-water mixture was pre-homogenized with an Ultra Turrax Polytron (Kinematica AG, Switzerland) for 2 min and homogenized with a two-stage lab homogenizer Ariete (NS1001L-Panda, Niro Soavi, Parma, Italy) at 310 bar (wherein the second stage was 30 bar).

Rice starch-o/w emulsions were prepared by mixing the stock o/w emulsions, stock starch dispersions, and a stock emulsifier (WPI) solution at calculated ratios. The ratio of mixing



is calculated to yield same emulsifier concentration in the aqueous phase for all the rice starch-o/w emulsions. All rice starch-o/w emulsions were freshly prepared on the day of measurements and kept under gentle stirring until the moment of further analysis.

### 6.2.2.3 Rice starch in emulsion-filled gels

The previously described stock native and gelatinized starch dispersions, as well as stock o/w emulsion and emulsifier solution were mixed with gelatin solutions at different ratios. Gelatin concentration in the aqueous phase was 4% (w/w). Gelatin was hydrated in water at room temperature for 2 h and then was heated in a waterbath at 80 °C for 30 min. Afterwards the molten gelatin was cooled to 30 °C by running tap water around the container. The gelatin solution was mixed with the stock native or gelatinized rice starch dispersions at calculated ratios. The starch-gelatin mixtures were stirred for about 2 min and cooled to 25 °C before mixing with emulsions and emulsifier solutions. The mixtures were allowed to form gels in plastic syringes as described previously (Liu *et al.*, 2015). The gels were stored in the refrigerator at 4 °C for 20 h and then were kept in thermos-cabinet at 20 °C for 2 h prior to further analysis.

## 6.2.3 Sample characterization

### 6.2.3.1 Particle size measurements

Droplet size distribution of the stock o/w emulsion, particle size of the native and gelatinized rice starch dispersions were measured using a Mastersizer2000 (Malvern Instruments Ltd., Malvern, UK). The 40% (w/w) stock o/w emulsion was first diluted 5 times by water before adding to the Mastersizer chamber. Samples were added into the chamber until an obscuration rate was reached between 10% and 12%. Volume-to-surface diameter ( $D_{3,2}$ ) was used as the parameter characterizing mean droplet size or particle size. A refractive index of 1.480, 1.472, and 1.330 were used for the fat droplets, starch particles (both native and gelatinized) and the aqueous phase, respectively. An absorption index of 0.01 was used for both fat droplets and starch particles. The calculation of droplet and particle size distribution was based on a general purpose model distribution with fitting accuracy above 98%.

### 6.2.3.2 Determination of gelatinization temperature

The gelatinization temperature of rice starch was determined using differential scanning calorimetry (DSC) (DSC Q1000, TA instruments, USA). About 30 mg of native starch dispersion (starch : water = 1 : 3, w/w) was weighed in an aluminum pan (Tzero Pans, TA instruments,

USA) and an empty pan was used as an inert reference. The starch sample was heated from 5 °C to 110 °C at a heating rate of 5 °C/min. The peak value ( $T_g$ ) of the endothermal curve was used as the gelatinization temperature.

#### 6.2.3.3 Microstructure

Scanning Electron Microscope (SEM) (Phenom tabletop G2 pure Scanning Electron Microscope, Phenom-World BV, The Netherlands) was used to determine the microstructure of native rice starch particles in dry form. Native starch was directly placed on a carbon adhesive pad that was stuck to a stub ( $d = 12.7$  mm). The stub was then placed in a standard sample holder (Phenom-World BV, The Netherlands). SEM image magnification was adjusted with a rotary knob.

Confocal Laser Scanning Microscope (CLSM) (LEICA TCS SP5, Leica Microsystems CMS GmbH, Mannheim, Germany) equipped with an inverted microscope (Leica DM IRBE) was used to determine the microstructure of native and gelatinized rice starch dispersions and emulsion-filled gels. Samples were stained with 0.2% (w/w) Nile blue aqueous solution. The objective lens used was HCX PL APO 63x/1.20 Water CORR CS (Leica). Digital images were acquired at a resolution of 1024 x 1024 pixels.

#### 6.2.3.4 Viscosity

Viscosity of native and gelatinized rice starch dispersions and rice starch-o/w emulsions were determined using a rheometer (Physica MCR 501/302, Anton Paar, Graz, Austria) at 20 °C. A concentric double gap cylinder geometry (DG26.7/TI) was used for all samples. The shear rate was increased and decreased in logarithmic steps recording 10 data points per decade from 1 to 1000  $s^{-1}$  and then from 1000 to 1  $s^{-1}$  in 10 min. Each sample was measured in duplicate. The average values of viscosity as a function of shear rate were reported.

#### 6.2.3.5 Large deformation properties

Large deformation properties of the rice starch-emulsion-filled gels, including Young's modulus, stress at fracture, strain at fracture, and recoverable energy were determined using an Instron universal testing machine (M5543, Instron International Ltd., Belgium). Uni-axial compression tests were performed using plate-plate geometry. Gel samples were cylindrical with the diameter of 26.4 mm and the height of 25 mm. Both the top and bottom of the gel surfaces were lubricated with a thin layer of paraffin oil to prevent friction between sample and plate during compression. For the determination of Young's modulus, stress at fracture,

and strain at fracture, measurements were performed at a constant compression speed of 1 mm/s up to 20% of their original height. For the determination of recoverable energy, measurements were performed at a constant compression and decompression speed of 1 mm/s up to 80% of their original height. All measurements were conducted on eight specimens and the average values were reported.

#### 6.2.3.6 Tribological properties

The tribological properties of native and gelatinized rice starch dispersions, rice starch-o/w emulsions and rice starch-emulsion-filled gels were determined using an optical tribological configuration (OTC) (Dresselhuis *et al.*, 2007; Liu *et al.*, 2016). Samples were sheared in the tribometer between a tribo-pair consisting of a glass plate and a flat bottom PDMS probe (Sylgard 184 Dow Corning, USA; mixing ratio of PDMS : cross-linker = 10 : 1; diameter = 6 mm). The surface of the PDMS probe is the upper surface. The load applied between the upper surface and the sample was set to 0.5 N. During each measurement, the glass plate oscillated over a distance of 16 mm at an increasing oscillation speed from 10 to 80 mm/s with incremental steps of 10 mm/s. Each speed was kept for 10 oscillation cycles. Friction force was determined during the movement of the glass surface. Friction force at each speed was reported as the average value of 10 cycles. For each measurement, a new probe was used and the glass surface was cleaned with water and ethanol. All the measurements were conducted at 20 °C in triplicate.

## 6.3 Results and Discussions

### 6.3.1 Characterization of rice starch particles and oil droplets

The DSC heat flow curve (figure not shown) indicated that the gelatinization temperature range of rice starch was 61 – 76°C with the peak value of 68.5°C, which was consistent with reported data (Purcell *et al.*, 2014). The gelatinization of starch is an endothermic process that involves the breakdown of intermolecular bonds of starch molecules and binding of water (Ghiasi *et al.*, 1982; Wang & Copeland, 2013). The fully gelatinized starch can be completely disrupted by mechanical shear, resulting in loss of the granular shape and dissolution of the starch molecules in the continuous phase (Kasapis *et al.*, 2009). In order to guarantee sufficient granule swelling, but avoid complete melting or dissolution of the starch granules, in this study the gelatinized starch was prepared by heating the native starch aqueous dispersion at the gelatinization temperature (68.5 °C) for 20 min.

The mean particle size ( $D_{3,2}$ ) of native and gelatinized rice starch in the aqueous dispersion was  $4.7 \pm 0.2 \mu\text{m}$  and  $7.8 \pm 0.1 \mu\text{m}$ , respectively. The sizes of both the native and gelatinized rice starch particles shown in the microscopy images (Figure VI - 1B and C) are consistent with the sizes determined with the light scattering. Figure VI - 1A and B show that the size of native starch particles in dry form is similar to that in aqueous dispersion. Native rice starch particles have irregular, polyhedral shape with well-defined edges. This is in agreement with literature (Ali *et al.*; Ohtani *et al.*, 2000; Rani & Bhattacharya, 1995) (Wani *et al.*, 2012). Figure VI - 1C shows that the gelatinized starch granules swelled and became more irregular shaped than native starch. The granular structure of most rice starch particles was generally maintained. This is due to the existence of amylose that gave some rigidity to the starch particles during swelling (Rani *et al.*, 1995). No obvious granule disintegration was observed. The mean oil droplet size ( $D_{3,2}$ ) of the o/w emulsions was  $1.9 \pm 0.03 \mu\text{m}$ .

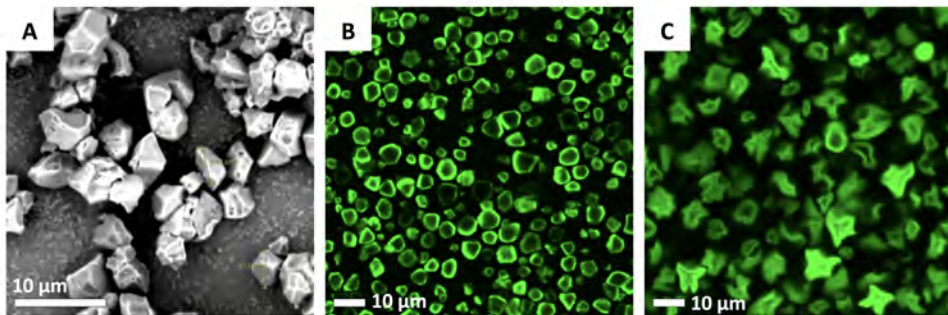


Figure VI - 1 Microstructure of rice starch. A) SEM image of native rice starch particles (dry powder); B) CLSM image of native rice starch dispersion; C) CLSM image of gelatinized rice starch dispersion (native rice starch heated in water at  $68.5^\circ\text{C}$  for 20min). Scale bar in all images represents  $10 \mu\text{m}$ .

### 6.3.2 Liquid food model systems: dispersions and rice starch-o/w emulsions

#### 6.3.2.1 Viscosity

Figure VI - 2A, B and C show the flow curves of native rice starch in water (0% oil) and in oil emulsions (5 and 20% oil). The viscosity of native rice starch dispersions increased with increasing starch and oil concentration. In general, the dispersions displayed low viscosities and Newtonian flow behavior. The 6% native starch dispersion with 20% oil displayed slight shear thinning behavior.

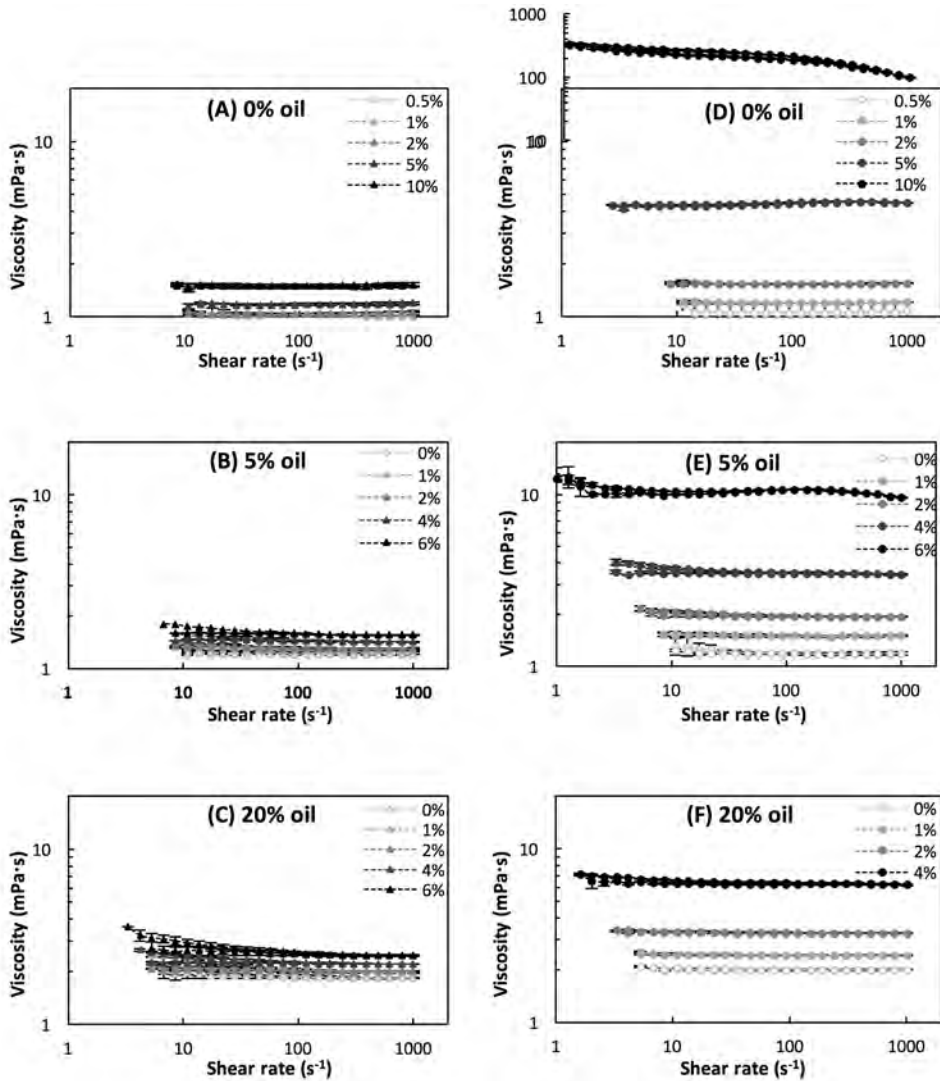


Figure VI - 2 Viscosity of native rice starch (A, B, C) and gelatinized rice starch (D, E, F) in 0, 5 and 20% of o/w emulsions as a function of shear rate. Legend in the figures indicates concentration of rice starch (w/w). Error bars represent the standard deviation of duplicate measurements.

Figure VI - 2D, E and F show the flow curves of gelatinized rice starch in water (0% oil) and in o/w emulsions (5 and 20% oil). Viscosity increased 5 to 200 times after the starch was gelatinized. During starch gelatinization, amylose and amylopectin molecules inside the starch particles leaches out simultaneously with the swelling of the granules (Lii *et al.*, 1996). Both molecular and granular structures of the gelatinized rice starch contribute to

the increase in viscosity. It is reported that in early heating stages, the increase in viscosity is mainly due to the leached-out molecules, while in later heating stages, the continued increase in viscosity is due to the swelling of the granules as well as the interaction between the leached-out materials and the granules (Lund & Lorenz, 1984). At high starch concentrations, the leached-out molecules may form gel-like network and the swollen granules are embedded in such a continuous matrix (Lii *et al.*, 1996).

### 6.3.2.2 Tribology

#### Rice starch dispersions

Figure VI - 3 shows that for both native and gelatinized rice starch, with increasing starch concentration, the friction curves shift to the right to higher viscosity  $\times$  shear rate values due to an increase in viscosity. The friction curves also shift upwards with increasing starch concentration, indicating an increase in friction coefficient. The shape of the friction curves suggests that the lubrication of native rice starch is in the boundary and mixed lubrication regime (Figure VI - 3A), and the lubrication of gelatinized rice starch is in the mixed lubrication regime and is almost entering the hydrodynamic lubrication regime (Figure VI - 3B). The difference in the friction curves may indicate different frictional behaviors for these two types of starch systems.

The native rice starch particles increase friction coefficient between the tribo-pair surfaces probably due to their polyhedral shape, sharp edges, and relative hardness (not measured in this study). A similar findings have been reported for custards with added particles, where irregular shaped particles resulted in higher friction than spherical particles (de Wijk *et al.*, 2005). Spherical microparticulated whey protein particles (with similar size range as native rice starch) reduce friction as they can roll easily over each other and between the surfaces of the tribo-pair (Liu *et al.*, 2016). In contrast, the native rice starch particles indirectly increased the surface roughness and increased interlocking between surface asperities, similar to a 3-body abrasion effect (Gates, 1998; Stachowiak & Stachowiak, 2001). Due to the irregular shape, with increasing concentration of native starch particles, agglomeration may happen during the shear (Bi *et al.*, 2011; Eliasson & Bohlin, 1982; Fall *et al.*, 2012). Agglomeration can increase both the apparent size and irregularity of the particles, which can indirectly increase surface roughness (i.e. asperity interlocking) and therefore increase friction (Bhushan, 1998; de Wijk *et al.*, 2005; Khan *et al.*, 2014; Santamarina *et al.*, 2004) (Scheme VI - 1). The shift towards the boundary lubrication regime at high native starch concentration confirms this mechanism.

Gelatinized starch particles are softer and more deformable due to absorption of water and losing part of their crystalline structure (Eliasson, 2006; Steeneken, 1989). Therefore, under the shear occurring in the tribometer, neither ball bearing nor 3-body abrasive effect was likely to be responsible for its frictional behavior. The soft and deformable nature of gelatinized starch particles can fill and flatten the asperities, thus leads to smoother surface contact. In addition, the gelatinized rice starch particles form a thicker layer and separate the tribo-pair surfaces due to the increased viscosity. This explains the complete absence of boundary lubrication regime in the friction curves. Amylose and amylopectin leach out from the swollen starch granules during the heating process of gelatinization (Aguilera & Stanley, 1999; Lii *et al.*, 1996). These leached out polymers, especially the amylopectin, could increase the stickiness of the starch dispersions (Adhikari *et al.*, 2001; Iturriaga *et al.*, 2006). The stickiness of starch may resist the relative motion of the tribo-pairs due to molecular adhesion and therefore increase the friction (Adhikari *et al.*, 2001; Myshkin *et al.*, 2005; Wu *et al.*, 2015). In the end, the impact of gelatinized rice starch on friction is a combined effect of 1) starch particles that smoothens the surfaces; 2) increase of viscosity that separate surface contacts; and 3) sticky molecules that resist the surface movement (Scheme VI - 2).

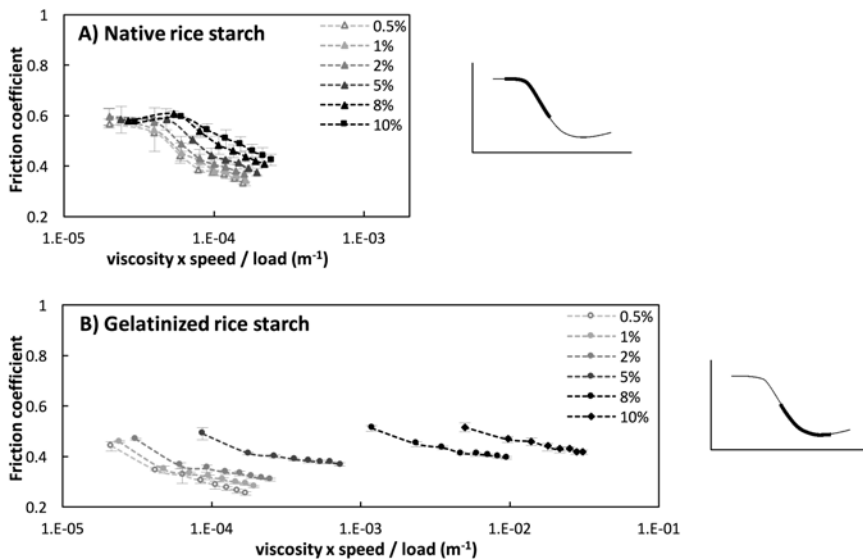
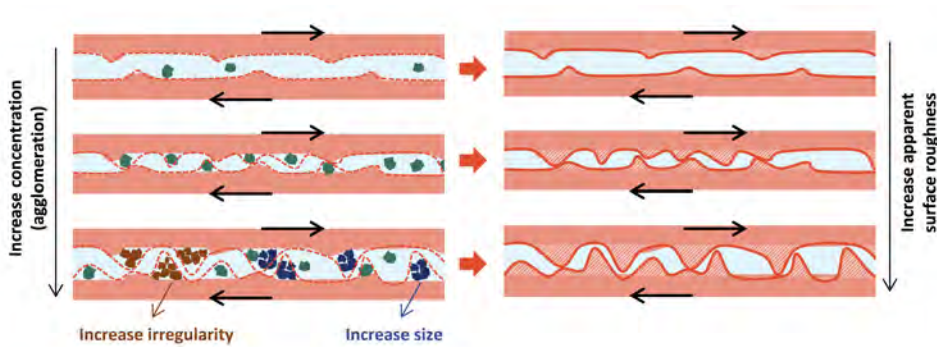
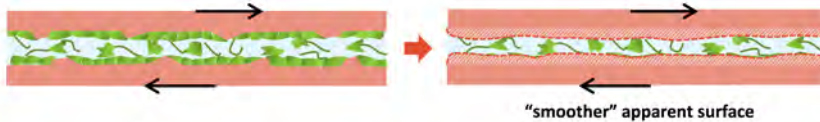


Figure VI - 3 Friction coefficient of A) native rice starch; and B) gelatinized rice starch at various concentrations as a function of the friction parameter (viscosity x speed / load). Error bars represent the standard deviation of triplicate measurements.



Scheme VI - 1 Friction behavior of native rice starch dispersions



Scheme VI - 2 Friction behavior of gelatinized rice starch dispersions

Rice starch in o/w emulsions

With increasing concentration of native rice starch in presence of 5 and 20% oil droplets, friction coefficients increased (Figure VI - 4A & B). This behavior is similar to the native rice starch dispersions (Figure VI - 3A), where native starch particles behave as a 3-body abrasive material. The oil droplets were expected to reduce the friction due to ball bearing or plate-out (Schmid *et al.*, 1996). However, this is not observed (comparing Figure VI - 3A with Figure VI - 4A). Furthermore, we observe an extended boundary lubrication regime in the friction curves of native rice starch at high concentrations (> 4%) in presence of oil droplets, in comparison with the friction curves of native rice starch in water. Probably the presence of oil droplets promoted agglomeration of native rice starch particles. With increasing amount of oil droplets from 5 to 20%, the friction coefficients decreased. The increase in oil content also leads to higher chance of agglomeration of rice starch particles, thus the boundary lubrication regime appeared even at low rice starch concentration (> 1%).

Figure VI - 4C and D show that with increasing concentration of gelatinized rice starch in presence of 5 and 20% oil droplets, friction curves shift towards higher friction parameter values due to the increase in viscosity. Despite this shift, friction curves also shift downwards, indicating the decrease of friction coefficient (within the same lubrication regime) with increasing starch concentration. To better visualize this decreasing trend of



friction coefficient, friction coefficients of gelatinized rice starch in the boundary lubrication regime are plotted against the starch concentration (Figure VI - 5). This decreasing trend is opposite from the results shown in Figure VI - 3B, where increasing gelatinized rice starch concentration leads to increasing friction coefficient. A possible explanation is that the coverage of oil droplets on the surface reduced the surface area to which the leached-out starch molecules adhered to, thus the friction caused by sticky starch molecules was eliminated. Instead, the soft starch particles and the increase of viscosity contributed to reducing the friction due to smoothing of the surface and better separation of the surfaces.

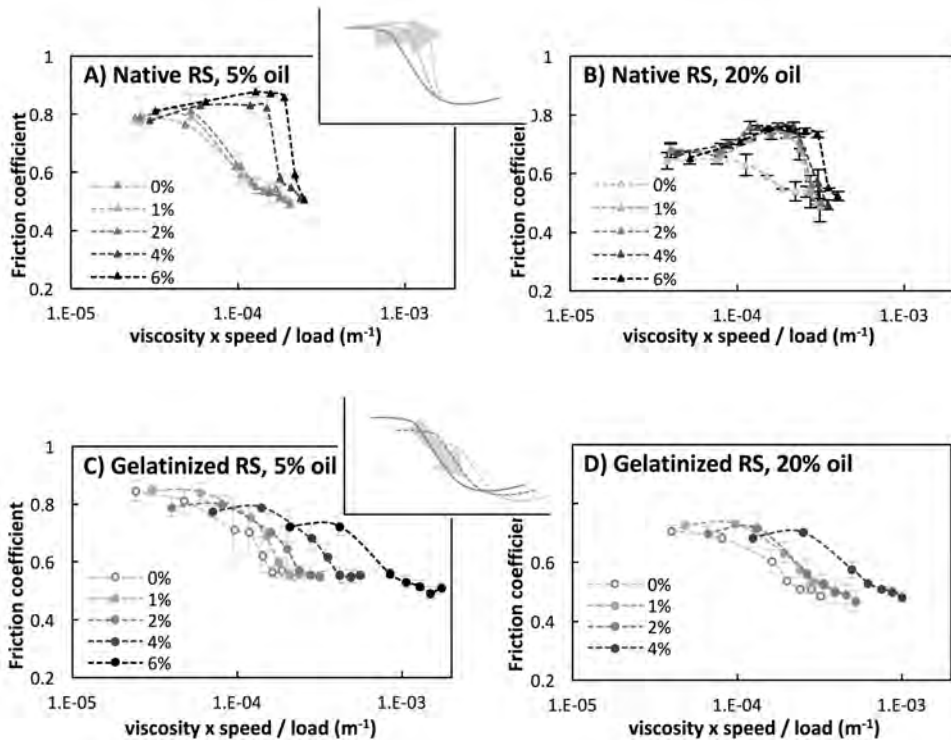


Figure VI - 4 Friction coefficient of A) native rice starch-o/w emulsions with 5% oil; B) native rice starch-o/w emulsions with 20% oil; C) gelatinized rice starch-o/w emulsions with 5% oil; and D) gelatinized rice starch-o/w emulsions with 20% oil at various concentrations as a function of the friction parameter (viscosity x speed / load). Error bars represent the standard deviation of triplicate measurements.

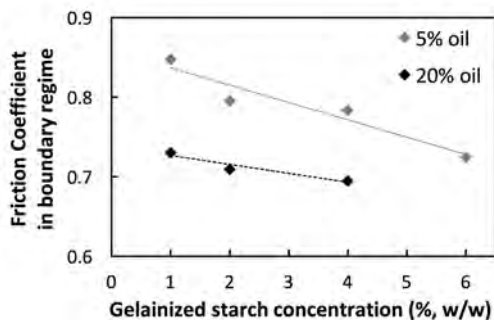


Figure VI - 5 Average friction coefficients (in boundary regime) of gelatinized rice starch-o/w emulsions with 5% and 20% oil (same data as Figure VI - 4C and D).

### 6.3.3 Semi-solid food model systems: rice starch-emulsion-filled gels

#### 6.3.3.1 Large deformation properties

Figure VI - 6 shows the effect of native and gelatinized rice starch particles on the mechanical properties of emulsion-filled gels. For gels that only contain oil droplets, fracture stress increased and fracture strain decreased with increasing oil content, and Young's modulus increased two-fold with increasing oil content from 0 to 20%. This is in line with previously reported results for bound oil droplets (Liu *et al.*, 2015; Sala *et al.*, 2007a). Regardless of the oil content, with increasing concentration of native rice starch from 0 to 6%, Young's modulus of emulsion-filled gels increased. This indicates that native rice starch particles behave as active fillers in the gelatin gel matrix and the modulus of native rice starch is higher than the modulus of the gelatin matrix (Eliasson *et al.*, 1982; van der Poel, 1958; van Vliet, 1988). The native rice starch particles showed no obvious impact on the fracture stress, fracture strain, and recoverable energy of the emulsion-filled gels, which supports the fact that starch particles act as bound fillers.

Regarding gelatinized rice starch particles, the Young's modulus of the gels increased with increasing starch concentration as a general trend (Figure VI - 6A). As rice starch particles became softer after gelatinization due to the absorption of water, it is possible that the modulus of gelatinized starch particles is smaller than the gelatin matrix. This could explain the decrease of the Young's modulus at low gelatinized starch concentration (Eliasson *et al.*, 1982; van der Poel, 1958). Further increasing concentration of gelatinized rice starch leads to dense packing of particles and more particle interactions (Figure VI - 7). The densely packed gelatinized starch particles may contribute to building gel network apart from

the gelatin matrix (Steeneken, 1989). In addition, the increasing amount of leached-out starch molecules may also reinforce the network of gel matrix. Increasing concentration of gelatinized rice starch decreased the fracture stress, fracture strain, and recoverable energy of the gels. In this case, due to the softness and relatively large particle size of gelatinized rice starch, they may create vulnerable regions inside the gelatin matrix and therefore behave as a structure breaker.

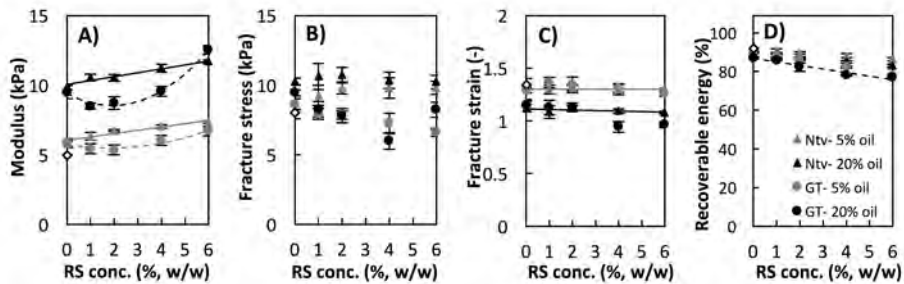


Figure VI - 6 Large deformation properties emulsion-filled gels containing native rice starch ( $\blacktriangle\blacktriangle$ ) or gelatinized rice starch ( $\bullet\bullet$ ) at different concentrations. A) Young's modulus; B) Fracture stress; C) Fracture strain; D) Recoverable energy.  $\diamond$  = 4% gelatin (gel matrix without oil or starch); symbols in grey = 5% oil; symbols in black = 20% oil. Error bars represent the standard deviation of eight measurements. Lines are shown in selected figures to guide eyes.

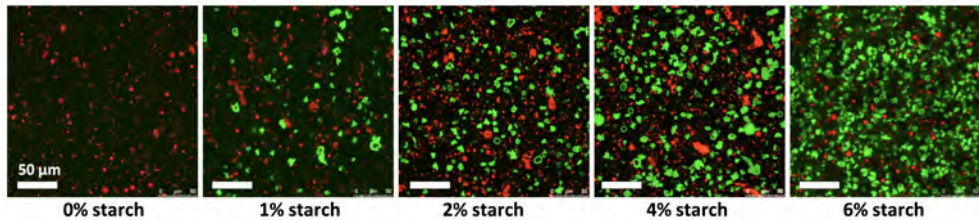


Figure VI - 7 CLSM images of gelatinized rice starch at different concentrations (w/w) in emulsion-fill gels containing 5% fat. Scale bar in all images represents 50  $\mu\text{m}$ .

### 6.3.3.2 Tribology

It is not possible to measure the true viscosity of emulsion-filled gels, therefore the friction coefficients of these semi-solid gel samples were plotted against sliding speed alone, instead of the friction parameter. Figure VI - 8A and B show the friction curves of native rice starch in emulsion-filled gels containing 5 and 20% oil. Independent of the oil content, friction coefficients of native rice starch in emulsion-filled gels increase with increasing native

rice starch concentration. This behavior is similar to native starch in liquid emulsions. In general, the friction coefficient of the gelled system is lower than in the liquid emulsions, and no boundary lubrication regime is observed in the friction curves of the gels. A possible explanation is that the thick gelatin matrix can separate the surfaces better than liquid emulsions. The gelatin matrix may, to some extent, mask the abrasive effect of native starch particles.

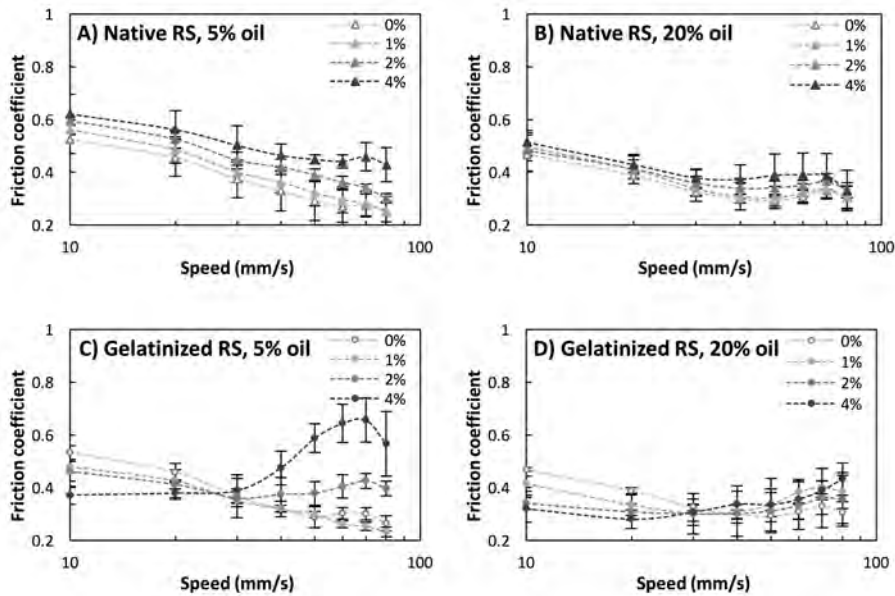


Figure VI - 8 Friction coefficient of emulsion-filled gelatin gels containing A) native rice starch and 5% oil; B) native rice starch and 20% oil; C) gelatinized rice starch and 5% oil; and D) gelatinized rice starch and 20% oil as a function of the sliding speed. Legend in the figures indicated the concentration of rice starch (w/w). Error bars represent the standard deviation of triplicate measurements.

Figure VI - 8C and D show the friction curves of gelatinized rice starch in emulsion-filled gels. Independent of the oil content, the friction coefficient behaves differently for low (about 0-1%) and high (2-4%) concentrations of starch. In particular, at low starch concentrations, the friction coefficient will tend to decrease as speed increases, while the situation is reversed for high starch concentrations. This indicates that with increasing concentration of gelatinized rice starch, the shape of the friction curves will tend to shift from mixed lubrication to hydrodynamic lubrication. This shift is probably caused by the increase of viscosity (although not measured) of the sheared gel as concentration of gelatinized rice

starch increases. For Figure VI - 8B and D (higher oil content), the trend is similar to that of lower oil content. In general, the friction coefficients tend to be lower, and the variability (especially with respect to starch concentration) is smaller.

## **6.4 Conclusions**

We investigated the tribological properties as well as rheological properties of native and gelatinized rice starch in liquid o/w emulsions and semi-solid emulsion-filled gelatin gels. For both native and gelatinized rice starch dispersions, friction coefficient increases with increasing concentration. This may give an indication that the fat-mimicking functionality of rice starch is probably not due to their contribution to lubrication, but due to other factors such as contributions to thickness and melting. Native rice starch increases friction probably due to its irregular shape and particle agglomeration at high concentration that indirectly increase surface roughness and asperity contacts. This can also be concluded from the appearance of an extended boundary lubrication regime in the friction curves. Gelatinized rice starch increases friction probably due to the stickiness of leached starch polymers. In the case of rice starch-o/w emulsions, oil droplets could reduce the friction caused by stickiness of gelatinized rice starch. Appearance of the hydrodynamic lubrication regime in the friction curves of gelatinized rice starch is due to the increased viscosity and sliding speed. The tribological behaviors of rice starch-emulsion-filled gels are more complex than liquids due to the bulk properties and breakdown properties of gel matrix. We conclude that morphology and surface properties of the starch particles, bulk and breakdown properties of matrices are the main factors that determining the tribological profile of food model systems.



# Chapter 7

## General discussion

## 7.1 Introduction

Food products need to meet a multitude of requirements for nutrition, sensory expectation and perception. The general approach to achieve an optimal balance of nutrition and sensory aspects is to understand how the macroscopic properties of foods relate to the molecular properties of their ingredients. Important in this respect is the role of structure in terms of molecular properties and macroscopic properties. It is described by many researchers, that in particular rheological and tribological properties are important for sensory perception.

In view of the above, the aim of this thesis is to unravel relations between structural elements, rheological and tribological properties during food breakdown, and the sensory perception of foods. More specifically, the aim is to understand the properties of food particles embedded in liquid and semi-solid food model systems in relation to the rheological and tribological properties and the relevance for sensory perception (Figure VII - 1). By varying food particles in size, morphology, deformability and stability, a more fundamental understanding of the influence of food particles properties on rheological and tribological properties of model systems is gained (**chapter 2, 4, 6**). By tuning the interactions between the food particles as well as between the other structural elements present (**chapter 2, 4, 6**), food model systems with different breakdown behavior, rheological and tribological properties are obtained. By analyzing the perception of fat-related and textural attributes of these designed model systems (**chapter 2, 3, 5**), relationships between the sensory perception and the physical properties of foods, as well as the interrelations between different sensory attributes are identified. In this general discussion, major considerations regarding the structural elements, as well as methodologies for characterizing physical and sensorial properties are given first. Following this, the main results and their interrelations are summarized and discussed. Finally, implications of the current findings are given, along with main conclusions and recommendations for future work.



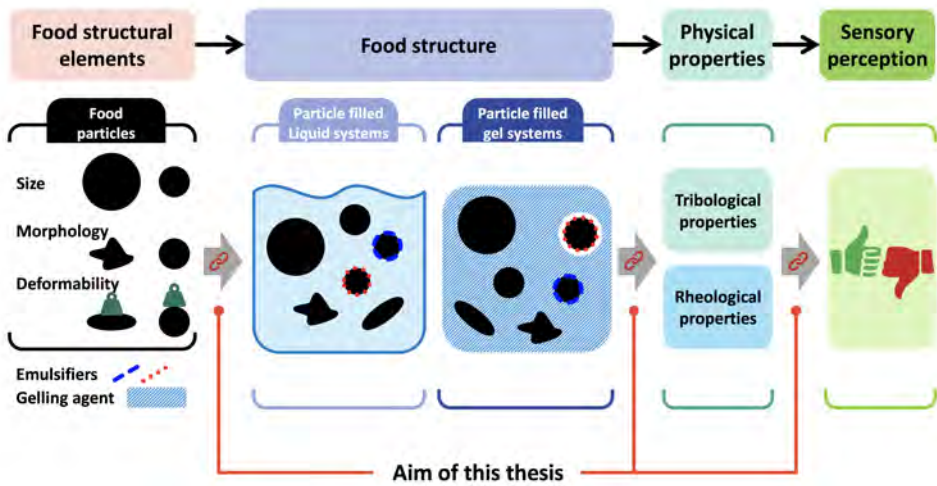


Figure VII - 1 Approach of the thesis: Linking properties of food structural elements to sensory perception via rheology and tribology

## 7.2 Major considerations

### 7.2.1 Considerations of food structural elements: matrix and particles properties

This thesis concerns both liquid and semi-solid food model systems, intending to resemble a wide range of real food products. The food model systems are particles dispersed in liquids and particle-filled semi-solid gels. Particles chosen in this thesis cover the range of the three major macronutrients from our diet: fats, proteins and carbohydrates. Particles that vary in size, deformability and morphology were chosen. These model systems allowed us to investigate the relationships between physical properties and the sensory perception of foods. Aspects of both the matrix and the particle fillers are discussed in the following.

#### 7.2.1.1 Semi-solid gel matrix

Modifying gel matrices can be used to design textural properties of particle-filled gels. Various gelling agents with diverse viscoelastic properties can be used, such as gelatin, whey protein, carrageenan, agar, etc. In **chapter 2**, gelatin was chosen to form a polymer gel, and in a continuation study, acid-induced whey protein isolate (WPI) gel was chosen as a particulate gel. Fat droplets were embedded within the matrix. The interactions between

the matrix and the fat droplets can be tuned by the choice of emulsifier that stabilizes the droplets (Sala *et al.*, 2007a). When using gelatin as a gel matrix, WPI and Tween 20 were used to design droplets bound and unbound to the gel matrix, respectively. When using WPI as a gel matrix, droplets stabilized with whey protein aggregates were bound to the matrix. A suitable emulsifier that makes droplets unbound to a stable WPI gel was not found. In subsequent chapters of this thesis, gelatin was used as a matrix since it enabled us to design both bound and unbound type of droplets.

#### 7.2.1.2 Fat droplets

In many types of processed foods, fats exist in the form of droplets and are mostly embedded in an aqueous phase. The amount of fat droplets in food affects the appearance, and the textural and sensory properties (Chung *et al.*, 2015). The first consideration was the concentration of fat droplets. In this thesis, the amount of ingredients is always described in weight percentage. The concentration of fat droplets among commonly consumed dairy and meat products ranges from less than 1% (skim milk, low fat yoghurt, etc.), to 10% (low fat cheeses), 22% (whipped cream), 27% (sausages), and 35% (full fat cheeses) (all in w/w). In this thesis, to represent a large variety of low fat and full fat foods, the fat content of the studied samples was in the range of 0 to 36% for the liquid food model systems and in the range of 0 to 20% for semi-solid systems.

The size of fat droplets in foods ranges from 0.1  $\mu\text{m}$ , such as fat globules in milk, to 50 - 100  $\mu\text{m}$ , such as fat droplets in meat batter (Mulder & Walstra, 1974; Zhang *et al.*, 2013). In processed food emulsions, different sizes of fat droplets can be produced using different homogenization pressures. Since the impact of droplet size on the physical and sensory properties of emulsion-type foods has been extensively studied (de Wijk *et al.*, 2005; K. H. Kim *et al.*, 2001; McClements *et al.*, 1993), in this thesis, the fat droplet size was not varied, but was kept around 1.5  $\mu\text{m}$  for all emulsions and emulsion-filled gels.

In **liquid emulsions**, it is described by several researchers that coalescence of fat droplets occurring during oral processing led to a decrease of friction and an increase of perception of fat-related attributes (Benjamins *et al.*, 2009; Chojnicka-Paszun *et al.*, 2012; Dresselhuis *et al.*, 2008c). The sensitivity of emulsion droplets towards coalescence, i.e. the stability of emulsion droplets, can be modified by changing their sizes or their solid fat content (SFC), adding unsaturated mono-glyceride, and by changing the emulsifier type and concentration (Benjamins *et al.*, 2009; Dresselhuis *et al.*, 2008c). For the **emulsion-filled gel** systems described in **chapter 2**, the sensitivity towards coalescence was modified by changing the SFC. Benjamins *et al.* (2009) reported that emulsions with medium solid fat contents (26 -

36%, at 20 °C) showed the highest degree of partial coalescence. In this thesis, the range of SFC was chosen as 4 - 48% (20 °C). Enhanced fat coalescence in emulsion-filled gels was observed with increasing SFC (**chapter 2**), but this did not influence the perception of fat-related sensory attributes. This was probably due to the decreased difference in SFC at oral temperature (Figure VII - 2), i.e. the melting of the solid fat used in the study at oral temperature. Since SFC did not influence fat perception, in **chapter 3 - 6**, the SFC was not varied. Since unsaturated mono-glycerides were speculated to have a separate mechanism to increase fat perception other than promoting coalescence (Benjamins *et al.*, 2009), it was not considered in this thesis. Two types of emulsifier were selected in this thesis, namely WPI and Tween 20, mainly with the purpose of designing different emulsion-filled gel structures.

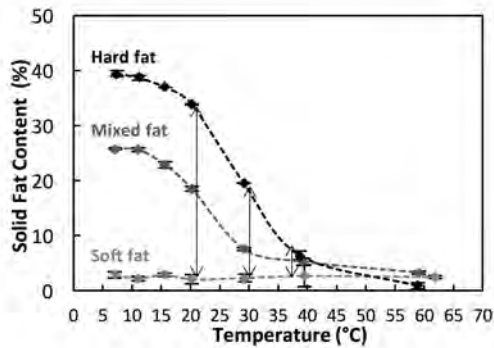


Figure VII - 2 Solid fat content (SFC) of emulsions as a function of temperature.

#### 7.2.1.3 Protein particles

Evidence for ball-bearing lubrication of spherical microparticulate protein particles was obtained (**chapter 4**). Microparticulate protein particles can be derived from different protein sources (Table I - 1, chapter 1). **Chapter 4** focuses on microparticulated whey protein (MWP), which is based on whey protein concentrate from milk. Other types of protein particles can be based on protein-polysaccharide complex formation, such as zein with carboxymethylcellulose, and egg white protein with xanthan or with pectin. Although zein has the benefit of mimicking the hydrophobic nature of fat droplets, flavor problems have been described (Roller *et al.*, 1996), and therefore zein particles were not used.

The size of MWP particles is reported in the range of 0.1 – 3  $\mu\text{m}$  (Cheftel *et al.*, 1993; Gaull, 1991). In this thesis, the MWP particles size distribution was bimodal with two main peaks at 0.4 and 7  $\mu\text{m}$ . Very large particles up to 30 - 50  $\mu\text{m}$  were also observed. These large

MWP particles enable us to observe the effect of particle size on the sensory perception of particles (**chapter 5**).

#### 7.2.1.4 Starch particles

In **chapter 6**, following the same concept of MWP, carbohydrate particles with micrometer size and spherical shape could also have the potential to provide lubrication in food products. In this thesis, native micro-granular starch particles were investigated. A wide range of small micro-granular starches is available in nature, such as parsnip (1 - 6  $\mu\text{m}$ ), rice (2 - 8  $\mu\text{m}$ ), amaranth (1 - 2  $\mu\text{m}$ ), cow cockle (0.3 - 1.5  $\mu\text{m}$ ), Quinoa (0.5 - 3  $\mu\text{m}$ ) (Jane *et al.*, 1994; Lindeboom *et al.*, 2004; Wani *et al.*, 2012). These native micro-granular starches are, however, not perfectly spherical. It is possible to obtain spherical micro-granular starch particles by separating them from larger granules, such as small wheat starch particles (2 - 10  $\mu\text{m}$ ) in whole flour (Malinski *et al.*, 2003). Since this thesis is interested in understanding how the morphology of the particles influences their lubrication properties, rice starches were chosen to compare with the spherical fat and spherical MWP particles. In addition, the deformability of rice starch was changed through gelatinization (**chapter 6**).

## 7.2.2 Considerations of methodologies

### 7.2.2.1 Large deformation mechanics and rheology

In order to understand how rheological and mechanical properties of foods can be related to sensory perception, suitable methods to measure these properties are required. It has been shown that the mechanical properties of semi-solid foods under large deformations can be linked to the sensory perception of food texture (Luyten *et al.*, 1992; Montejano *et al.*, 1985). To determine the large deformation properties, in **chapter 2 - 6**, the uniaxial compression test was employed since it resembles part of the human mastication process (Roudaut *et al.*, 2002; Sala, 2007) and provides real material properties. In this thesis, a constant compression speed of 1 mm/s was chosen, which was reported to be relevant for in-mouth compression of semi-solid foods between the upper palate and the tongue (Rosa *et al.*, 2006). The gel samples were compressed by 80% of their original height to ensure fracturing. In the case of measuring recoverable energy, the gel samples were compressed by 20% of their original height. The gel sample size ( $\sim 15$  g) in this thesis is relevant for large bite size of semi-solid foods (Zijlstra *et al.*, 2009).

In this thesis, true stress and true strain at the fracture point were used to quantify the fracture properties. Hardness and firmness were often found to correlate to fracture stress,

and brittleness was often found to correlate to fracture strain (Devezeaux de Lavergne *et al.*, 2015; Gwartney *et al.*, 2004; Sala *et al.*, 2008; Xiong *et al.*, 2002). The slope in the linear viscoelastic region of the stress/strain curve was used to calculate the Young's modulus, which is in turn related to firmness and stiffness of the gels (Foegeding *et al.*, 2011). The recoverable energy of gels was measured as it may correlate to crumbliness (van den Berg *et al.*, 2008; van den Berg *et al.*, 2008b).

Unlike the breakdown properties of semi-solid gels, liquid foods exhibit flow behavior. In **chapter 3 - 5**, the flow behavior of the liquid food models was determined as a function of shear rate. Viscosity is often well related to the perception of several sensory attributes such as thickness and creaminess (Kokini *et al.*, 1977; Malone *et al.*, 2003; Richardson *et al.*, 1989).

#### 7.2.2.2 Tribology

General concepts of tribology are presented in the introduction of this thesis (**chapter 1**). Tribological results of this thesis (**chapter 2, 4 - 6**) were reported as so-called Stribeck curves, which were determined by plotting the friction coefficient as a function of sliding speed. According to the shape of the Stribeck curves, the friction behavior of most of the food model systems studied were in the boundary and mixed lubrication regimes. As viscosity plays an important role in the mixed regime, the so-called "friction parameter" (defined as the product of speed and viscosity divided by normal load) was used in order to make the impact of viscosity and normal load explicit (**chapter 4 - 6**). By using the friction parameter, we are better able to identify the impact of other factors, e.g. particle properties. Since it is not possible to define a shear viscosity for a gel sample, the contribution of viscosity was only considered for the liquid model systems.

Several types of tribometers are used in the area of food research. The differences among these types are often regarding the type and speed of the surface movements, contact area between the surfaces, and the surface materials. Mini Traction Machine (PCS Instruments) and Anton Paar tribology equipment are two types of commercially available tribometers. The MTM consists of a ball (or ring) and a disk, each independently driven and is able to produce a sliding/rolling contact in a desired ratio. This slide-to-roll ratio was found to affect the friction (Chojnicka *et al.*, 2008), thus often a fixed ratio is set during the friction measurements. The MTM has been successfully applied for studying foods and hydrocolloids in many studies (Chojnicka-Paszun *et al.*, 2014a; Garrec *et al.*, 2013; Malone *et al.*, 2003; Mills *et al.*, 2013). Regarding the gelatin gels in this thesis, the gel samples could not hold on to the disk due to the centrifugal force generated from the high-speed rotation. In addition,

the sticky gelatin pieces formed big lumps, which interfered with the entrainment of the sample between the surfaces as well as with the movement of the ball. The Anton Paar tribology setup consists of a rotating ball as the upper surface loaded onto three probes that are arranged radially slanting outward  $45^\circ$  from the shaft. Since the probe material used was PDMS, which is relatively soft, we encountered a problem of not being able to guarantee that the three probes were perfectly symmetrically aligned at the same height. If this alignment does not exist, the contact surface area and the forces between the ball and each probe may differ, leading to erroneous friction values. This was confirmed by the observed different degrees of wear on the probe surfaces after the tribology measurements. Due to its non-horizontally aligned geometry, it was not suitable for the measurement of particle dispersions that were prone to sedimentation. Optical tribological configuration (OTC), developed by a team of TIFN researchers, consists of two surfaces sliding over one another in a parallel movement (Dresselhuis *et al.*, 2008c). This was claimed to have the advantage of being more similar to the tongue-palate parallel reciprocating sliding movement than the circular rolling/sliding movements of other tribometers. Another advantage of OTC is that the surfaces can be easily adapted. For example, Dresselhuis *et al.* (2008b) applied pig tongue as the upper surface to mimic in-mouth lubrication, and thin glass as the bottom surface to allow for microscopic observations. Although the range of testing speeds of OTC is limited (10 – 100 mm/s), the speeds are quite relevant for oral conditions, especially for fat-related sensory perceptions (Malone *et al.*, 2003). Considering these factors, this thesis employed the OTC for the measurement of friction coefficient (**chapter 2 - 6**). The combination of OTC with a Confocal Laser Scanning Microscope (CLSM) allowed us to observe the coalescence of fats under shear in the contact between the two surfaces of the tribometer (**chapter 2**).

Although one of the highlights of OTC is the possibility of applying oral tissue, due to limited availability, difficulty in preservation as well as the large individual differences between the oral tissues (Dresselhuis *et al.*, 2008b), polydimethylsiloxane (PDMS) was chosen as the tribology surface (upper surface of the tribometer) in this thesis. PDMS has been applied by many other research groups (Bongaerts *et al.*, 2007; Dresselhuis *et al.*, 2007; Garrec *et al.*, 2013). The PDMS probes prepared in this thesis have an elastic modulus about two orders of magnitude higher than that of the tongue. It would have been closer to real oral conditions if the PDMS probes were made as deformable/soft as the tongue. However, the deformation of the surface can induce friction due to a ploughing effect (Bhushan, 2013). Since this thesis only focuses on the different lubrication behavior of food samples, such a soft surface was not used. PDMS probes were prepared by molding them in surface-sanded plates, yielding asperities of tens to hundreds of micrometers, similar to that of the tongue (Uemori *et al.*, 2012). The bottom surface of the tribometer was made of a thin slide of

glass, which represents the upper palate and enables us to observe the microstructure of samples immediately after the measurements.

The choice for the value of the normal force is based on the consideration of gaining a desired contact pressure relevant for oral conditions. The PDMS probe used in this study has a flat surface area (if not considering the actual surface area of all asperities) of 28 mm<sup>2</sup>. Thus, by choosing a fixed normal force of 0.5 N, a contact pressure of 17.6 kPa was achieved, which was in the range of contact pressures of the tongue (13 - 30 kPa) during swallowing (Pouderoux & Kahrilas, 1995). The wear of the surface was found to be negligible at these conditions (Dresselhuis *et al.*, 2007).

In order to maintain the designed gel structure and SFC, the friction measurements in this thesis were conducted at room temperature (20 °C), as the gelatin matrices and the solid fat melt at oral processing temperature (37 °C).

#### 7.2.2.3 Sensory

Three types of sensory tests are often used to characterize the texture properties of food products: discrimination tests, acceptance/liking tests, and descriptive analysis tests. In **chapter 2 & 5**, Quantitative Descriptive Analysis (QDA) tests were used. The QDA test can provide a complete quantitative sensory description among a set of products, allowing the investigation of the impact of product properties on the specific attribute, as well as the interrelations among different attributes. QDA does not reveal the evolution of sensory attributes over time, while progressive profiling is a dynamic sensory test that allows evaluation of the intensity of selected attributes at different time intervals during oral processing. In **chapter 3**, progressive profiling was used to evaluate the perception of the mouthfeel attribute fatty at 33, 66, and 100% of the full mastication time, and the intensity of the corresponding afterfeel attribute fatty at five time intervals within 180 s after expectoration. This helps to link the dynamic perception of fat to the formation and clearance of fat fractions in oral coatings, which can be measured instrumentally. In **chapter 5**, two groups of iso-viscous liquid emulsions containing different amount of fat droplets and protein particles were prepared. Since the aim is to analyze whether the difference between particle lubrication can have an impact on the perception of iso-viscous liquids, a paired-comparison test (two-alternative-forced-choice-method) was conducted by a naive panel, in addition to a QDA conducted by a trained panel.

### 7.3 Summary of the main results

#### 7.3.1 Rheological properties of food model systems

##### 7.3.1.1 Large deformation properties of semi-solid gels

Table VII - 1 summarizes the influence of different types of food particles on the large deformation properties of semi-solid gels. Increasing concentrations of fat droplets (stabilized with 1% WPI) increased the modulus and fracture stress of emulsion-filled gels, indicating that droplets were bound to the matrix. In this case, the recoverable energy of the gel was influenced minimally with increasing droplet concentration. Opposite observations were found for fat droplets stabilized with Tween 20. In this case, the fat droplets were unbound to the gel matrix (they behaved as inactive fillers). Increasing SFC in the fat droplets was found to increase the modulus (in agreement with results from Oliver *et al.*, (2015)), while decreasing the fracture strain of the gels. This would be related to the presence of solid fat crystals that could make the droplets behave like partially bound droplets.

**Table VII - 1 Influence of each component on large deformation properties of gelatin gels in chapter 2 – 6. Symbols in the table: ↗ increase; ↘ decrease; — no influence; ? no trend/relation was found.**

Emulsifier	Particle content	Modulus	Fracture stress	Fracture strain	Recoverable Energy	Interaction with matrix
WPI	Fat ↗	↗	↗	↘	—	Bound
	Solid fat ↗	↗	?	↘	—	Bound
Tween 20	Fat ↗	↘	↘	—	↘	Unbound
	Solid fat ↗	↗	↘	↘	↘	≈ Bound
MWP	MWP ↗	↗	↗	—	(slightly)↘	Bound
	Native starch ↗	↗	—	—	(slightly)↘	entrapped
	Gelatinized starch ↗	↗	—	—	(slightly)↘	entrapped

MWP particles are expected to have electrostatic interactions with the matrix, like the WPI-stabilized emulsion droplets do. Increasing concentrations of MWP particles in emulsion-filled gels led to an increase in modulus and fracture stress, indicating that MWP particles were bound to the matrix. No obvious influence of MWP was found on the fracture strain. MWP decreased the recoverable energy of the gels only slightly, which suggests again that the particles were bound to the matrix.

Increasing concentrations of native and gelatinized rice starch increased the modulus of the



emulsion-filled gels, suggesting that these starch particles were effectively bound to the matrix. As starch is not expected to interact with the gelatin matrix, it is more likely that these starch particles were entrapped in the matrix due to their irregular shape. Solubilized starch molecules that were leached out from the gelatinized rice starch might also have an impact on gel strength (not studied in this thesis). No obvious influence of starch particles was found on the fracture strain of the gels, and they decreased the recoverable energy of the gels only slightly, which correspond to entrapped unbound particles.

#### 7.3.1.2 Viscosity of liquid systems






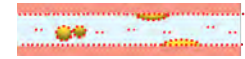



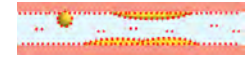

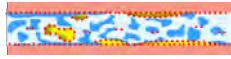


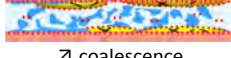













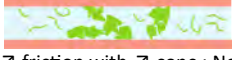






All particles were found to increase the viscosity upon increasing concentration. At concentrations below 8% (w/w), fat droplets, MWP particles and native rice starch granules in water yielded thin Newtonian fluids ( $\eta < 10$  mPas). The viscosity of these particle dispersions only increased to a minor extent with increasing particle concentration. At concentrations higher than 8% (w/w), slight shear thinning behavior was observed at lower shear rates. Gelatinized rice starch dispersions displayed higher viscosities than native ones, due to the larger particle size and irregularities, as well as due to leached-out starch molecules in the continuous phase. The viscosity of emulsions at equal fat content did not change by the type of emulsifier used.

### 7.3.2 Tribological properties of food model systems

Table VII - 2 summarizes the tribological properties of different food particles in both liquid and semi-solid food model systems. In the case of fat droplets, emulsions and emulsion-filled gelatin gels showed lower friction coefficients than water and gelatin gels, indicating that fat droplets had a lubrication effect in both liquid and semi-solid matrices. Increasing the fat content of emulsions and emulsion-filled gels led to a decrease in friction. In liquid systems, droplets that were stabilized with Tween 20 showed lower friction than those stabilized with WPI, indicating that the type of emulsifier can affect lubrication. In gel systems, unbound droplets (stabilized with Tween 20) were released more easily than bound droplets (stabilized with WPI) from the gel matrix, thus higher coalescence and lower friction were observed for unbound droplets. Increasing the solid fat content (SFC) of the fat droplets resulted in more droplet coalescence and thus lower friction.

In the case of MWP particles, MWP reduced friction in both liquid and semi-solid gel systems, but less effectively in semi-solid gels than in liquids. In the systems where emulsifier Tween 20, MWP, fat droplets and gel matrix were all present, the friction behavior became too complex to unravel, since all these structural elements contributed to it.

Table VII - 2 Summary of the tribological results in this thesis. Symbols in the table: ↗ increase/higher; ↘ decrease/lower.

		Liquid system	Semi-solid gel system
Chapter 2 & 3	Fat	 fat (WPI)  ↘ friction than water	 bound  ↘ friction than gelatin gel
	Fat	 fat (Tween 20)  ↘ friction than WPI emulsion	 unbound  ↗ fat release & coalescence; ↘ friction than bound
	Fat	 ↗ fat content  ↘ friction with ↗ fat content	 ↗ fat content  ↘ friction with ↗ fat content
	Fat	 ↗ SFC Not studied	 ↗ SFC  ↗ coalescence ↘ friction with ↗ SFC
Chapter 4 & 5	Protein + fat	 MWP  ↘ friction than water; friction reduction restricted at high conc.	  ↘ friction than gelatin gel; Less efficient than in water;
	Protein + fat	 MWP + fat (WPI) Further ↘ friction with existence of fat	  Further ↘ friction with existence of fat
	Protein + fat	 MWP + fat (Tw20) Further ↘ friction with existence of fat and Tween 20	  Complex lubrication effect
Chapter 6	Rice starch + fat	 Native  ↗ friction with ↗ conc.; Appearance of boundary regime	Not studied
	Rice starch + fat	 gelatinized  ↗ friction with ↗ conc.; No boundary regime	Not studied
	Rice starch + fat	 native + fat (WPI) ↗ friction with ↗ conc.; extended boundary regime	  ↗ friction with ↗ conc.;
	Rice starch + fat	 gelatinized + fat (WPI) ↘ friction with ↗ conc. in boundary regime, ↗ friction in mixed and hydrodynamic regimes	  ↘ friction with ↗ conc. at low speed; ↗ friction at high speed

In the case of starch particles, both native and gelatinized starch dispersions increased friction upon increasing concentration. Friction curves of native starch showed boundary lubrication regimes, while those of gelatinized starch showed mixed lubrication regimes. This indicates different friction behaviors between the native and gelatinized starches. The existence of fat droplets in the system eliminated part of the boundary friction caused by native starch particles.

### **7.3.3 Sensory properties**













In this thesis, the sensory properties of the previously discussed particle-filled liquids and gels were studied. The results are summarized in Table VII - 3. Sensory attributes were generated by trained panels. The perception of textual attributes and fat-related attributes was correlated to the physical properties of the model systems and oral processing.

The large deformation properties of the semi-solid gels and the viscosity of the liquids were correlated to the perception of texture attributes, such as mouthfeel firm, elastic, brittle, and spreadable. Regarding the emulsion-filled gels, a stronger perception of mouthfeel firm and elastic was always associated with higher modulus and fracture stress, and gels with higher fracture strains were perceived as less brittle and more spreadable. Regarding the liquid MWP-o/w-emulsions, the perception of thickness did not correlate with the viscosity of the sample.

Regarding emulsion-filled gels without other food particles, the fatty and creamy perception were related to a decrease in friction caused by unbound fat droplets or by increasing fat droplet concentration, but were not related to the decrease in friction that was caused by increasing SFC. Inclusion of MWP particles in liquid emulsions decreased the friction, which was expected to increase fat-related perception. However, MWP particles increased the perception of roughness and dryness, which suppressed creaminess. Thus, the perception of fat-related attributes is not simply related to the reduction of friction by particles in liquid systems. In particle-filled gels, the perception of rough and dry from particles was masked by the gel matrix, thus the perception of creaminess was not suppressed by roughness, and in this case, the perception of creaminess showed a correlation to a decrease in friction.

The perception of fattiness, which was correlated to the fat fraction deposited on the tongue, increased with increasing mastication time, and decreased with increasing clearance time after expectoration of the emulsion-filled gels.

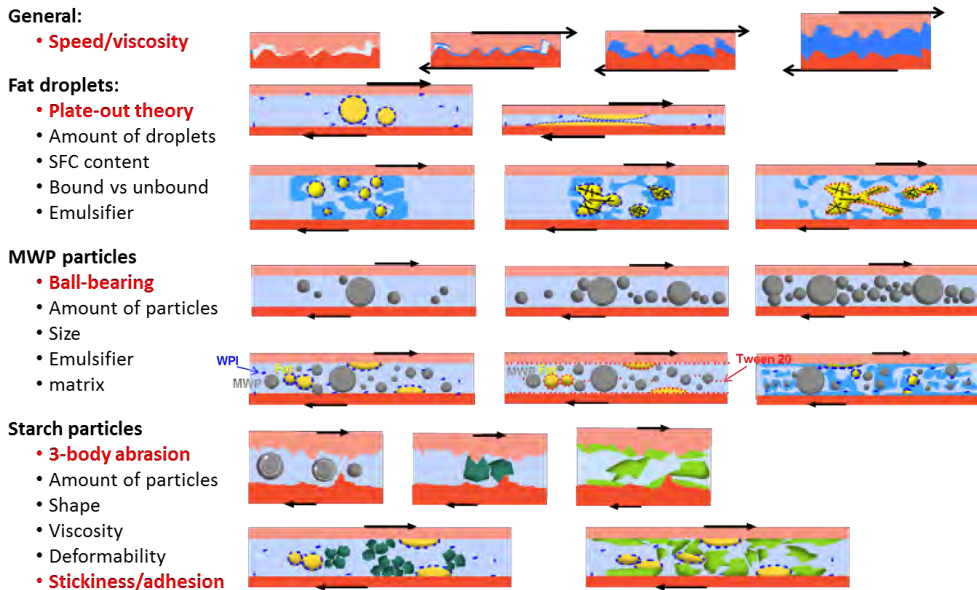
**Table VII - 3 Summary of the sensory results in relation to rheological and tribological properties. Symbols in the table: ↗ increase; ↘ decrease; — no influence; ? no trend was found.**

Type of sample		Perception of textual attributes in relation to rheology	
Chapter 2	 Gels with unbound droplets	Modulus & Fracture stress ↘ Fracture strain ↗ (compared to bound)	Firm & Elastic ↘ Brittle ↘ Spreadable ↗
Chapter 2	 (Unbound) fat content ↗	Modulus & Fracture stress ↘ Fracture strain —	Firm & Elastic ↘ Brittle ↘ Spreadable ↗
Chapter 2	 (Unbound) SFC ↗	Modulus ↗ Fracture strain ↘	Firm & Elastic ↗* Brittle ↘ Spreadable ↗*
Chapter 5	 Fat — & MWP content ↗	Modulus ↗ Fracture stress ? Fracture strain —	Firm ↗ Elastic ↗* Crumbly & Spreadable —
Chapter 5	 Fat content ↘ & MWP content ↗	Viscosity —	Thickness ≠ Viscosity
Perception of fat-related attributes in relation to tribology			
Chapter 2 & 3	 Gels with unbound droplets	Fat deposition on tongue ↗ Friction ↘ (compared to bound)	Fatty ↗* Creamy ↗*
Chapter 2 & 3	 Fat content ↗	Fat deposition on tongue ↗ Friction ↘	Fatty ↗ Creamy ↗
Chapter 2	 SFC ↗	Friction ↘	Fatty — Creamy —
Chapter 5	 Fat content ↘ & MWP content ↗	Friction ↘	Fatty ↘ Rough, dry, powdery perception ↗ Creamy ↘
Chapter 5	 Fat content — & MWP content ↗	Friction ↘	Fatty — Creamy ↗
Perception of fat-related attributes in relation to oral processing			
Chapter 3	 Mastication time ↗	Fat deposition on tongue ↗	Fatty ↗
Chapter 3	 Clearance time ↗	Fat deposition on tongue ↘	Fatty ↘

\*Not significant at  $p < 0.05$ .

## 7.4 Discussions

### 7.4.1 Mechanisms underlying tribological behavior of multi-component food model systems



**Figure VII - 3 Overview of different mechanisms underlying tribological behavior of multi-component food and factors affecting the tribological properties**

The tribological behavior of food model systems between two sliding surfaces was investigated in this thesis (Figure VII - 3). The friction values were transferred into a so-called Stribeck curve to understand the major factors that determine the friction. The factors are categorized into two groups here: **Surface-related** and **bulk-related**.

Surface-related factors can be further classified as inherent factors (or testing parameters) and sample-induced factors. Inherent factors include the applied load on the surfaces, contact area between the surfaces, relative moving speed of the surfaces, as well as the physical and chemical properties of the surfaces (roughness, elasticity, hydrophobicity, etc.). The sample-induced factors are the physical and physicochemical properties of samples that alter surface properties, such as roughness of particles, adhesiveness of molecules, wetting properties of surfactants, etc. Bulk-related factors are mainly the rheological properties (i.e. viscosity) of the samples.

Bulk-related factors are more important when surfaces are completely separate. In this case, friction is caused by the forces resisting the relative motion of fluid molecules (viscosity). In a multicomponent system, the surface-related and bulk-related factors often affect each other, leading to a complex friction response. We are seeking explanations for the mechanisms underlying the observed differences in lubrication behaviors in food model systems based on these different factors.

#### 7.4.1.1 Influence of speed on lubrication

The division of lubrication regimes in a Stribeck curve is based on the shape of the curve, which shows the dependency on speed and viscosity. For particle and droplet dispersions that were studied in **chapter 4 - 6**, increasing the sliding speed from 10 to 80 mm/s caused a decrease in friction, indicating that they are in the mixed lubrication regime. In **chapter 4**, the fat droplets contained about 36% solid fat, which could promote droplet coalescence during shear. Thus, the observed effect of speed on friction might actually be the effect of coalescence due to increasing speed and the duration of the measurement. As is shown in Figure VII - 4, at fat contents below 20%, the friction force appears to depend only on the actual speed, and is independent of the speed history (i.e. acceleration or deceleration). In addition, as shown in Figure VII - 5, at fat contents below 20%, the friction force decreases with increasing speed, but remains the same at constant speed. In other words, the friction is independent of shearing time. Thus, it is concluded that for samples containing less than 20% fat, the decreasing friction force with increasing speed is only an effect of speed.

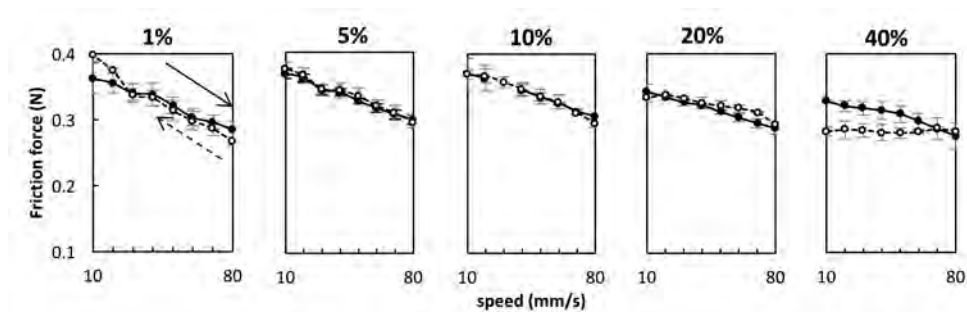


Figure VII - 4 Effect of increasing sliding speed (from 10 to 80 mm/s, filled symbols) and decreasing sliding speed (from 80 to 10 mm/s, open symbols) on the friction force of pork fat emulsions (SFC = 36%, stabilized with WPI) at concentrations from 1% to 40%.

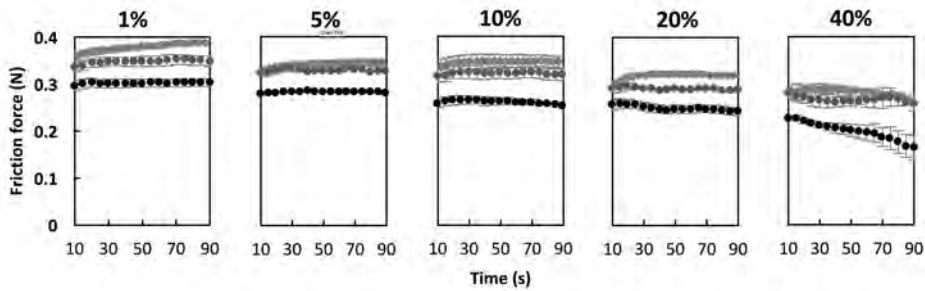


Figure VII - 5 Effect of sliding speed and time on the friction force of pork fat emulsions (SFC = 36%, stabilized with WPI) at concentrations from 1% to 40%. ● = 10 mm/s, ● = 40 mm/s, ● = 80mm/s.

However, as shown in Figure VII - 4, at a fat content of 40%, the friction curve of decreasing speed does not overlap with the curve of increasing speed. The friction appears to reach the lowest value at 80 mm/s, probably due to the extensive irreversible droplet coalescence. Furthermore, with the prolonged shearing time at 80 mm/s (Figure VII - 5), the friction of 40% fat decreases significantly. Thus, it can be concluded that at very high fat content (40%) and high SFC, the friction of an emulsion is influenced by speed-induced and time-induced coalescence. Since most liquid samples in this thesis contain less than 20% fat, the effect of time-induced structural changes on friction was neglected.

For gel samples that were studied in **chapter 2**, increasing the sliding speed from 10 to 80 mm/s induced a decrease in friction of the emulsion/particle-filled gels, indicating that they are in the mixed lubrication regime. It is shown in Figure VII - 6 and Figure VII - 7 that the friction force of emulsion-filled gels is independent of the speed history and time, indicating that the breakdown of gels in-between the sliding surfaces is probably completed soon after the beginning of shear. The actual amount of sample sheared between the tribo-surfaces during each measurement was small (< 100 mg), thus the breakdown of the gel could indeed occur in a short time (< 10s). Considering that the perception of tribology-related and fat-related attributes often occur after the solid food is finely chewed or molten in the mouth, while the process of food breakdown influences mostly rheology-related attributes, this thesis did not focus on the details of how the breakdown process affects the friction, but instead on how the broken gel influenced it. A slight decrease in friction is observed for gels containing 15% of high SFC droplets with prolonged shearing time, probably due to the increasing coalescence (Figure VII - 7).

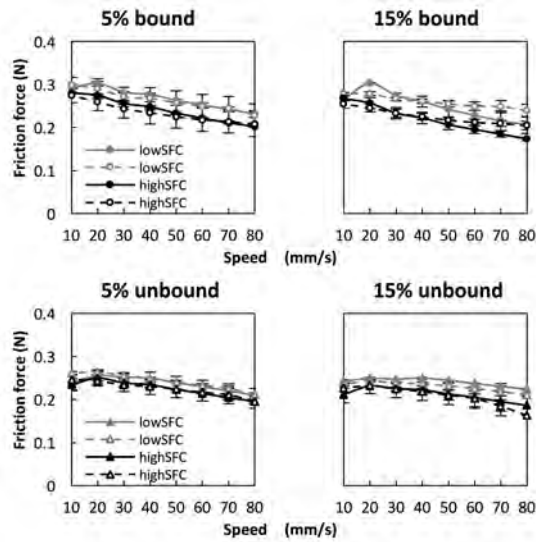


Figure VII - 6 Effect of increasing sliding speed (from 10 to 80 mm/s, filled symbols) and decreasing sliding speed (from 80 to 10 mm/s, open symbols) on the friction force of emulsion-filled gels. Legend: low SFC = 4%, high SFC = 36%; Bound droplets are stabilized with WPI, unbound droplets are stabilized with Tween 20.

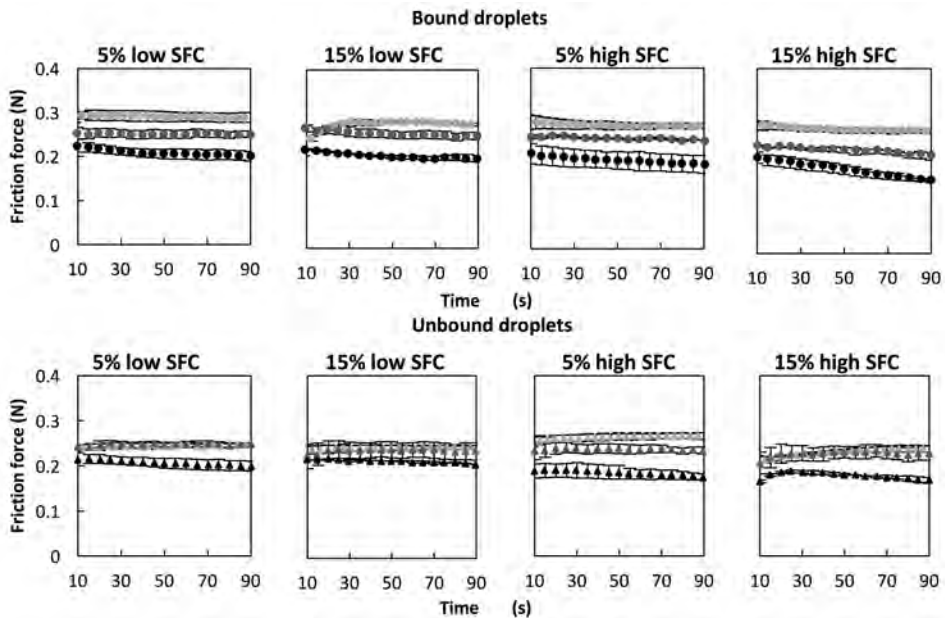


Figure VII - 7 Effect of sliding speed and time on the friction force of emulsion-filled gels. Legend: low SFC = 4%, high SFC = 36%; Bound droplets are stabilized with WPI, unbound droplets are stabilized with Tween 20; ● = 10 mm/s, ■ = 40 mm/s, ▲ = 80mm/s.



#### 7.4.1.2 Influence of concentration-related viscosity on lubrication

For the liquid samples in **chapter 4 - 6**, the contribution of viscosity to the friction coefficient was considered since the friction curves of all samples exhibited mixed lubrication regimes at the speed range studied. In the mixed lubrication regime, lubricants with higher viscosity can separate surfaces better, resulting in a lower friction. Increasing the concentration of MWP particles (**chapter 4**) and of fat droplets (Figure VII - 8A in section 7.4.1.4) leads to a decreasing friction coefficient. This might be an effect of increasing viscosity due to increasing concentration. After accounting for this contribution of viscosity to the friction coefficient, friction curves (as a function of viscosity x speed / load) with increasing particle concentration slightly shifted to the right while maintaining the decreasing trend in friction. This indicates that in the case of MWP particles and fat droplets, the decreasing friction coefficient with increasing concentration is not only due to increasing viscosity, but also due to other factors. These other factors can be for instance the ball bearing lubrication of spherical particles (section 7.4.1.5). In the case of gelatinized rice starch (**chapter 6**), the friction curves shift largely to the right with increasing concentration due to significant increase in viscosity. An increase in friction with increasing viscosity was observed, which is characteristic for the hydrodynamic regime. Since this observation applies to the mixed regime (cf. Figure VII - 3, chapter 6), it is concluded that there are other factors increasing friction, overruling the friction effect of viscosity. These other factors can be, for instance, the adhesiveness of the starch molecules (section 7.4.1.6).

#### 7.4.1.3 Lubrication of o/w emulsions: multiple mechanisms

The lubrication of o/w emulsions can be explained in terms of a few mechanisms. When it comes to lubrication during oral processing, the main mechanism is often ascribed to the coalescence of fat droplets in the mouth (Dresselhuis *et al.*, 2008c). The coalescence of fat droplets leads to the formation of oil films or film patches on the surface (**chapter 2 & 4**), following a so-called plate-out theory (Schmid *et al.*, 1996). The coalescence of fat droplets can be induced by a mechanical force under shear in a tribometer as well as in the mouth (Sarkar & Singh, 2012). The coalescence of fat droplets in the mouth might also be surface-induced (Dresselhuis *et al.*, 2008c) or due to droplet-saliva interaction (Hugo, 2005; Prinz *et al.*, 2005). The sensitivity of droplets towards coalescence can be increased by manipulating the emulsifier type and concentration, droplet size, and solid fat content (SFC) (Benjamins *et al.*, 2009; Dresselhuis *et al.*, 2008c; Liu *et al.*, 2015). **Chapter 2** showed that emulsions droplets containing high SFC (36%) displayed more coalescence (visualized under CLSM), leading to lower friction.

Fat droplets in **chapter 4** were sensitive towards coalescence since they have high SFC. In addition to this, droplets that were stabilized by Tween 20 could spread on the surface more easily compared to the WPI stabilized droplets, thus enhancing the plate-out effect. These results were in line with findings of Dresselhuis et al. (2008a) that protein-poor, unstable droplets spread more on solid surfaces than stable droplets. Thus, the adhesion and spreading of fat droplets could be important factors that influence lubrication.

Fat droplets in **chapter 6** were relatively stable against coalescence due to the absence of solid fat and their protein-stabilized surface. Small droplets are less deformable than coalesced large droplets. In this case, the main lubrication mechanism of the fat droplets was probably not plate-out. Small oil droplets that were trapped in surface asperities could behave similar to ball bearings. Stable oil droplets could roll over each other and contribute to increased hydrostatic pressure between the tribo-pair surfaces, thus reducing surface contacts and friction (Dresselhuis *et al.*, 2008c).

According to literature, other possible factors accounting for the lubricating properties of emulsion droplets, are their deformability, the viscosity of oil in the droplets, as well as phase inversion induced by changing the concentration or shear (de Vicente *et al.*, 2006; Schmid *et al.*, 1996). These mechanisms may play a role in the situations studied in this thesis, but to what extent is unknown.

#### 7.4.1.4 Lubrication of emulsifiers: adsorption at surfaces forming boundary film

Emulsifiers used in this thesis were WPI (aqueous phase) and Tween 20 (aqueous phase). In **chapter 2 & 3**, droplets stabilized with WPI were bound to the gelatin matrix, while droplets stabilized with Tween 20 were unbound. Unbound droplets were more easily released from the matrix upon shear than the bound droplets (observed with CLSM) and thus had a lower friction. In fact, this lowered friction could also be an effect of emulsifiers that were in the continuous phase. **Chapter 4** showed that the dispersed emulsifiers had a direct effect on the lubrication behavior of the particle-filled liquids and gels. Tween 20 stabilized emulsions showed lower friction coefficients than WPI stabilized emulsions (Figure VII - 8A). The Tween 20 solution showed lower friction coefficient than the WPI solution, and the WPI solution shows a higher friction coefficient than water (Figure VII - 8B). In comparison with the emulsions and emulsifier solutions, liquid fat shows the lowest friction in the hydrodynamic regime, indicating the formation of a thick fat film with high viscosity. The shapes of the friction curves of the emulsifier solutions shows a boundary lubrication regime at low speeds, which indicates that the effects of both WPI and Tween 20 are surface related. Tween 20 can adsorb on the tribometer surfaces and form a monolayer boundary film, preventing direct

contact between the surfaces (Chen *et al.*, 2002; Spikes, 1993). In contrast, adherence of protein at hydrophobic surfaces (PDMS in this thesis) was found to increase the boundary friction (Dresselhuis *et al.*, 2007). Thus, a difference in adsorption can explain the difference between Tween 20 and WPI. Mucins (glycoproteins) in saliva provide good lubrication in the oral cavity. In this case, the saliva-coated tongue surface is hydrophilic (Bongaerts *et al.*, 2007). It is concluded that the adsorption of an emulsifier layer at the surface influences the boundary lubrication. The actual details of this influence, although not investigated in this thesis, are likely dependent on the hydrophobicity of the surfaces and the physicochemical properties of the adsorbed molecules.

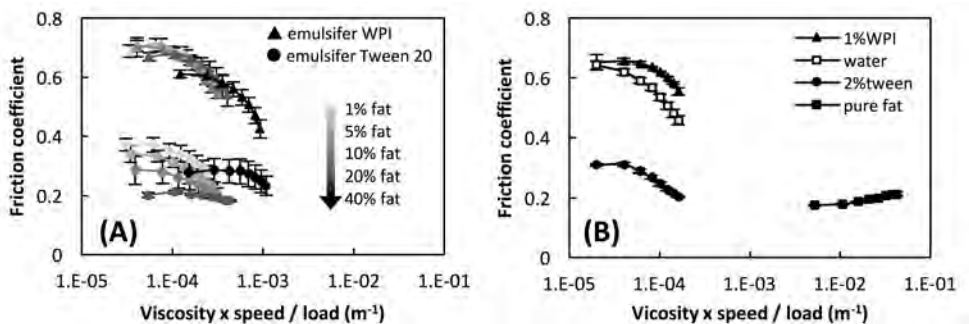


Figure VII - 8 Friction curves of A) fat emulsions (SFC = 36%) stabilized with 1% WPI or 2% Tween 20; B) emulsifier solutions, water and fat (liquid at 20 °C, SFC = 4%).

#### 7.4.1.5 Lubrication of micro-particles: effect of size, morphology, deformability and concentration

Microparticulated ingredients were used as fat replacers to mimic the physical shape and size of globular fat droplets. Microparticulated whey protein (MWP) particles could reduce the friction of both liquid and gel systems due to a ball-bearing lubrication mechanism (Chapter 4 & 5). Both the spherical shape of the particles and the asperity-particle size ratio were important in ball-bearing lubrication. The size of MWP studied in this thesis was in the range of 0.4 – 7  $\mu\text{m}$ , although some large particles up to 50  $\mu\text{m}$  were observed. The size of the asperities of the PDMS surfaces was in the range of approximately 10 - 100  $\mu\text{m}$  (observed using an optical microscope), resulting in an asperity-particle ratio of about 1 : 20. On one hand, small MWP particles could fill the asperity gaps, thus smooth the surface, and reduce the so-called asperity inter-locking. On the other hand, these spherical particles reduce the contact area between the tribo-pair surfaces, and change the local relative motion between the particle and tribo-pair from sliding to rolling. Chojnicka (Chojnicka *et al.*, 2008) showed that globular whey protein aggregates reduce friction with increasing concentration only

due to a viscosity effect, but not due to ball bearing. In their case, the size of asperities (Neoprene, 31  $\mu\text{m}$ ) was about 1000 times of the size of whey protein particles (30-80 nm), which very likely minimizes the ball bearing of particles and maximizes the viscosity effect.

Native rice starch particles were studied in **chapter 6** as another type of microparticulate ingredients to mimic fat droplets. Unlike the spherical MWP particles, native rice starch particles have irregular, polyhedral shapes with sharp edges. For this reason, they could not behave as ball bearings. Addition of native rice starch in o/w emulsions and emulsion-filled gels increased their friction coefficients. The shift in the lubrication regimes from mixed to boundary after adding native rice starch indicates increasing surface interactions. The irregular shapes and sharp edges of native rice starch effectively increase surface roughness and asperity contacts, similar to a 3-body abrasion effect.

Gelatinized rice starch particles studied in **chapter 6** were softer and more deformable than native rice starch particles. As a result, neither a ball bearing nor a 3-body abrasive effect was likely to be responsible for their frictional behavior. The soft and deformable nature of gelatinized starch particles allows them to fill and flatten rough surfaces, leading to a smoother surface contact. This explains the complete absence of boundary lubrication regimes in the lubrication curves of gelatinized starch dispersions, as well as their lower friction coefficients compared to native starches.

In **chapter 4 & 5**, increasing the concentration of MWP particles in water and emulsions led to a decreasing friction coefficient. At high concentrations, however, the friction coefficient reached a plateau. This is suggested to be due to the saturated deposition of particle layers at the surface (Garrec *et al.*, 2013). This indicates that the efficiency of ball bearing lubrication is concentration-dependent. In **chapter 6**, increasing the concentration of native starch particles in water and emulsions led to an increased friction coefficient and an extension of the boundary lubrication regime in the friction curves. This is due to the increased particle aggregation at higher concentrations, which increases the apparent asperity (cf. scheme 1, chapter 6), in addition to the effect of irregular shape.

#### 7.4.1.6 Friction properties of adhesive molecules

**Chapter 6** reported that the difference between the frictional behaviors of gelatinized and native rice starch dispersions was not only due to their different particle properties or due to viscosities. The increased friction coefficient of gelatinized rice starch with increasing concentration was ascribed to the adhesion of leached-out starch molecules to the tribo-pair surfaces. Molecular adhesion is considered as one of the major sources of friction (Myshkin

*et al.*, 2005). In the case of emulsions containing gelatinized rice starch, the coverage of oil droplets on the surface, or between the asperities, reduced the surface area to which starch could adhere. Therefore, adhesive friction was partially eliminated, making the lubrication effects of soft particles and effect of increasing viscosity become more dominant. Overall, the frictional behavior of gelatinized rice starch is a combined effect of particles, viscosity and adhesive molecules, and can be influenced by the lubrication of oil droplets.

#### 7.4.1.7 Lubrication of gel systems

One particular characteristic of gelled systems that liquid systems do not have is the breakdown of structure during oral processing. It is shown in Figure VII - 7 that the breakdown of gelatin gels was probably completed soon after the beginning of the tribology measurement, since no time dependency was observed in the friction curves. This also explains why the pre-sheared gels showed similar frictional behavior as whole piece gels. The matrix of all the gelled samples in this thesis was 4% gelatin. Overall, gelatin gels showed lower friction than water-continuous liquid samples (**chapter 2, 4 & 6**). Both the formation of a thin gelatin film on the surface, and the soft gelatin particles generated under shear in between the surfaces, are possibly responsible for this. For droplets and particles that were bound to the gelatin matrix, their lubrication or friction behavior was restricted compared to the unbound ones and in liquids, since they were less easily released from the matrix. **Chapter 4** showed that the ball bearing function of MWP in gels was less pronounced than in aqueous dispersions. **Chapter 6** showed that the boundary friction caused by irregularly shaped starch particles was eliminated in the gel systems. For fat droplets that were unbound, **chapter 2** showed that more oil was released from the gel and thus the friction was lower than the gels containing bound droplets.

The melting of gelatin could facilitate the formation of a thick lubrication layer. The melting temperature of gelatin used in this thesis was about 27 °C. Although gelatin was not likely to melt during the tribology measurements performed at room temperature (20 °C), it likely melts in the mouth during oral processing. This effect of a molten layer on lubrication, however, needs to be further investigated using a temperature-controlled set-up.

#### 7.4.1.8 Summary: combined lubrication mechanisms for complex systems

This thesis covers food model systems ranging from simple particle dispersions and emulsions, to particle-filled gels. As the systems become more complex, the complexity in lubrication behavior increases. Separately studying the lubrication behavior of simple systems helps in understanding their combined effect in multi-component and complex-structured systems.

The simplest systems studied in this thesis are water and fat. Being the continuous phase of all samples investigated, water alone shows mainly surface-related frictional behavior, while pure fat alone shows mainly bulk-related frictional behavior. When fat is dispersed into water as small droplets (i.e. in an emulsion), the effects of droplets (plate-out and ball bearing) and emulsifiers have to be considered. After introducing particles into the systems, the size, morphology, and deformability of the particles should also be taken into consideration. Other ingredients with particular characteristics, such as adhesiveness, also play important roles in lubrication. The gel matrix can be a lubricant itself, and can mask the lubrication or friction mechanisms of other components. In a multi-component system, these different mechanisms may enhance, suppress, or compensate each other. In the boundary and mixed lubrication regimes, these mechanisms, when listed roughly in order of significance, are the inherent properties of the tribo-pair surfaces and the measurement setting, emulsifiers that influence surface hydrophobicity, particles that influence surface roughness, plate-out of fat droplets, and the adhesiveness of the components. In the hydrodynamic lubrication regime, the influence of bulk properties (i.e. viscosity) is more important.

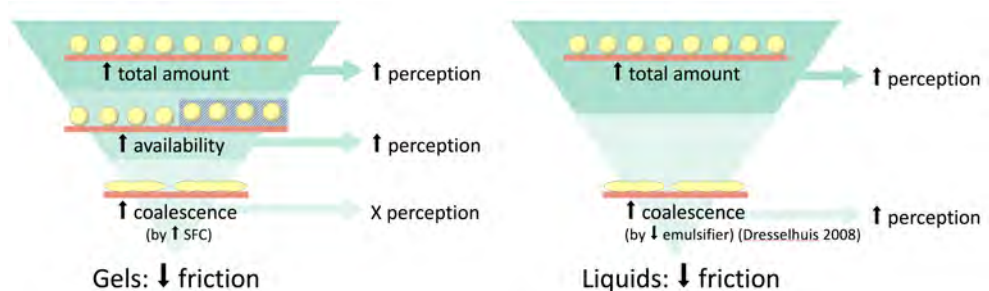
From the above discussion, it can be summarized that although the complexity of the lubrication behavior increases as the systems become more complex, the essence of all mentioned lubrication mechanisms is no more than the surface properties of the tribo-pair and bulk properties of the lubricant in between the surfaces. This gives an indication that in order to understand or predict the lubrication properties of a system, the primary task is to identify and classify all the factors that contribute to the surface and bulk properties, thereafter analyze what factors play key roles and what lubrication regime the system shows. After pinpointing the key factors, it should be possible to regulate the lubrication properties of the systems by balancing the factors that give positive and negative contributions to the lubrication.

## **7.4.2 Relating physical properties to sensory perceptions**

### **7.4.2.1 Influence of friction properties on fat-related perception**

It is commonly assumed that fat-related perception is primarily determined by the amount of fat (Kupirović *et al.*, 2012). This thesis suggests specifying this statement further, that it is primarily determined by the amount of fat that is in contact with oral surfaces. In general, this amount can be increased by three strategies (Figure VII - 9). The first, and also the most direct strategy, is to increase the total amount of fat. The second strategy is to increase the availability of fat, for example, as discussed in this thesis, by designing a gel containing

unbound droplets instead of bound droplets. This strategy is not relevant for liquid systems, since all the fat droplets are expected to be available anyhow. The third strategy is to increase the sensitivity of fat droplets towards coalescence, for example by using less emulsifier and increasing the SFC. The coalescence induces a larger contact surface between fat and the oral surfaces. All these strategies were effective in reducing friction as shown in this thesis (**chapter 2**) and as described in literature (Chojnicka *et al.*, 2009; Dresselhuys *et al.*, 2007). In all cases, except one, the decrease in friction is correlated with an increase in fat-related perception. The exception is in the case of SFC-induced coalescence in gels, where it is possible that the effect of SFC on coalescence is weakened due to melting of the solid fat under oral conditions (as compared to remaining solid in the tribometer at room temperature), therefore this coalescence effect could not be perceived (see also discussion in section 7.2.1.2). The amount of fat that is in contact with the oral surfaces (fat fractions deposited on the tongue) was quantified by using *in vivo* fluorescence methods in **chapter 3**. Results did show that the first two strategies (increase total fat amount and availability) effectively increased the fat fractions deposited on the tongue.



**Figure VII - 9 Three strategies to increase the amount of fat that is in contact with oral surfaces.**

In chapter 5, the sensory perception of MWP particles in liquid emulsions and emulsion-filled gels were investigated. Principal Component Analysis (PCA) of data obtained from QDA of the liquid samples shows a “fatty-rough” dimension (PC1) that explains most of the variations between the samples. This dimension is primarily driven by the content of fat droplets and MWP particles. The perceptions of mouthfeel (and afterfeel) fatty were positively correlated to fat content and negatively correlated to MWP content, and vice versa for the perception of mouthfeel (and afterfeel) rough/powdery/dry. Perception of mouthfeel (and afterfeel) creamy was correlated to fatty on PC1, but it could be distinguished from fatty on PC2. This indicates that perception of creamy and fatty are due to different mechanisms (Figure VII - 10). Results from QDA of the emulsion-filled gels show that the gel matrix masked the perception of rough/powdery/dry. In this case, both increasing the content of fat droplets

and MWP particles increased the perception of creamy. However, the perception of fatty is only related to the content of the fat droplets, and is independent from MWP.

Fatty perception is related to the main lubrication mechanism for fat emulsions, which is the formation of plate-out film patches (coalescence) on the surface. MWP particles reduce friction due to ball bearing, which is another lubrication mechanism for fat emulsions. Ball bearing lubrication fails to contribute to film formation and therefore fails to provide the fatty perception. Both the ball bearing lubrication and the plate-out film lubrication contribute to the perception of creamy. For MWP, only the ball bearing provided by small MWP particles contributes to creamy. Big particles with sizes above the oral detection threshold contribute to the perception of rough/powdery/dry, and suppress the perception of creamy. Interestingly, with respect to emulsion-filled gels, although the gel matrix restrained the ball bearing lubrication of MWP, it also masked the perception of rough/powdery/dry (compared to liquid emulsions). In the end, MWP in gels resulted in a positive effect on the creamy perception.

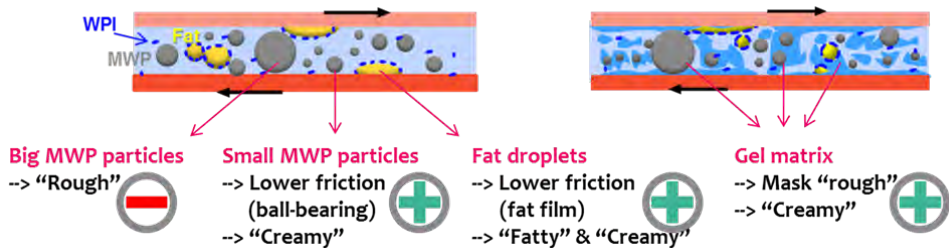


Figure VII - 10 Different lubrication mechanisms lead to different perception

A sensory study on rice starch was not performed in this thesis. Rice starch, either in native or gelatinized form was reported to be able to serve as natural fat replacer in foods including ice cream (Chigurupati *et al.*, 1992; Mason *et al.*, 2009), dressing (Bakal *et al.*, 1992), and sausages (Setser *et al.*, 1992), and were claimed to provide a smooth and creamy mouthfeel (Moldenhauer *et al.*, 1998). The fat mimicking function of rice starch is often ascribed to its small particle size in a similar way as with MWP (Joly *et al.*, 2009; Malinski *et al.*, 2003). However, this is different from what we expected, since the perception of irregularly shaped particles is often associated with rough, dry, gritty and powdery. **Chapter 6** showed that native rice starches increased friction due to their irregular shapes, and gelatinized rice starches increased friction due to stickiness. Therefore, the fat-like perception of rice starch cannot simply be related to the frictional properties as measured with a tribometer. This may be due to the fact that the oral surfaces are much softer than the PDMS in a tribometer, and that the surfaces are coated



by a layer of saliva. This layer might have prevented the friction of sticky gelatinized starch. Other than this, viscosity might also play a role. In the case of native rice starch particles, their irregular shape might not be so important in perception at the size level of rice starch (5  $\mu\text{m}$ ). In addition, it cannot be excluded that the effect of “melting” (from enzymatic degradation of starch in mouth) on perception might dominate the overall sensory perception of starch-containing products.

#### 7.4.2.2 Influence of rheological properties on fat-related perception

In **chapter 2**, two groups of emulsion-filled gels were prepared. In the first group, emulsion-filled gels had the same concentration of gelatin in the matrix, while containing different concentrations and types of fat droplets. These differences in fat droplet characteristics lead to differences in both large deformation properties and lubrication properties of the gels. For example, gels with unbound droplets released more fat droplets, showed lower friction values and lower gel modulus than gels with bound droplets. Gels with unbound droplets were perceived as fattier and creamier. In order to decouple the effect of large deformation properties from the effect of lubrication properties on the perception of fat-related attributes, **chapter 2** also studied a group of emulsion-filled gels that have equal large deformation properties (i.e. modulus) by changing the gelatin concentration, while keeping the other conditions the same as in the first group. It was found that for the modulus-controlled group, the link between lubrication properties of the gels and their fat-related perceptions was still observed. Thus, it was concluded that for the case of soft gels, fat droplets induce a fat-related perception that is independent of the modulus or other textural properties.

This conclusion at first seems to contradict findings from Devezeaux de Lavergne *et al.* (2015). These authors tested emulsion-filled gels made from mixtures of agar and gelatin with different fracture stresses and strains. They also included in the study design with both oil droplets being bound or unbound to the matrix. They found that gels with similar fracture properties did not show a difference in perception when containing bound versus unbound droplets. In their case, the large variation in fracture properties between gels probably overruled the effect of oil release. The different results of the two studies are attributed to the following differences. First, texture and fracture properties between the two studies were different. The Young's modulus of the emulsion-filled gels in this thesis was in the range of 5 - 15 kPa, while in the other study it was in the range of 3 - 300 kPa. The fracture properties of samples between the two studies were also largely different. Second, the gelling agents used in the two studies were different in perception and melting. The

results of the two studies combined indicate the importance of the gel structure design on their lubrication and sensory properties.

We note that in **chapter 5**, the perceived thickness could not be correlated to viscosity, but this is most likely due to the fact that the viscosities were low and did not differ much between the samples.

#### 7.4.2.3 Relating oral processing to perception

The effects of oral processing time and the follow-up consumption on fat fractions deposited on the tongue as well as fatty perception were investigated in **chapter 3**. Fat fractions deposited on the tongue increased rapidly in the first 2 s of mastication of emulsion-filled gels. Further increasing mastication time slightly increased the fat fraction deposited on the tongue, as well as the fatty perception. This indicates that the formation dynamics of the fat deposited on the tongue is a fast process. This also suggests that the first few bites are the most relevant for the formation of fat depositions on the tongue (regarding the samples that used in this study). This is to some extent in line with the previous discussion (section 7.4.1.1), that in a tribometer breakdown of gels or releasing of fat is a fast process. Fat fractions deposited on the tongue decreased rapidly during the first 15 s after the expectoration of the emulsion-filled gels. With further increasing clearance time up to 180 s, the fat fractions deposited on the tongue decreased gradually. The fat fractions were not completely cleared after 180s after expectoration. The fatty after-feel perception of the emulsion-filled gels also decreased with increasing clearance time. However, in contrast to the fat fraction clearance, there was no steep decrease in the fatty after-feel perception, but rather a smooth decrease. This is likely due to adaptation effects. Drinking water (which creates a water flow) has a stronger effect on the removal of fat depositions than masticating a gelatin gel. There are two possible opposite mechanisms regarding the removal of fat fractions by masticating a gelatin gel. First, the fat deposited on the tongue might easily be removed by the broken pieces of gelatin particles due to abrasion and mechanical rubbing. Second, the fat deposited on the tongue might be protected by the molten gelatin layer from removal caused by the mechanical rubbing of the oral surfaces. Considering these two possible mechanisms, the effect on the removal of fat depositions by masticating a gelatin gel might not be that strong after all. Although water is more effective than gelatin gel in removing the fat depositions on the tongue, the fat is still not completely removed. This gives an indication that studies that rely on the rinsing method to quantify coatings might underestimate the fat content in the oral coatings.

During oral processing, the temperature of foods is higher than the temperature at which

the physical measurements are conducted in this thesis. The temperature of the foods in the mouth depends on how fast the food is processed, as well as the size and the physical properties of the foods. Although the temperature of foods during oral processing was not measured in this thesis, it is assumed that the temperature of foods that are in direct contact with oral surfaces is probably very close to body temperature quickly after the first bites. In the case of emulsion-filled gelatin gels studied in **chapter 2 & 3**, in order to make fat droplets more available to contact the oral surfaces, the structure of emulsion-filled gels were designed by making droplets unbound to the matrix and by increasing the SFC in the droplets. The melting of gelatin and solid fat in the mouth is likely to have reduced the effect of the droplets being unbound versus bound, and the effect of coalescence induced by solid fat. In addition, the melting of gelatin contributes to the perception of creaminess. Other than the impact of temperature, in particular for starch-containing systems, under oral conditions, “melting” due to the salivary enzymes contributes to the perception of creaminess (Alting *et al.*, 2009). Thus, oral conditions obscure the understanding of effects of particle properties (studied at room temperature and in the absence of saliva) on the perception of creaminess.

## **7.5 Recommendations and implications**

### **7.5.1 Recommendations for future research**

The melting of gelatin under oral conditions changes the large deformation properties of the gelled systems. Because of this, the fracture properties of gels should only be relevant for the perception of textural properties within the first few bites. Actually, the fracture properties chosen (i.e. fracture stress and strain, and modulus) were indeed correlated to the texture perceptions during the initial phase of oral processing (Brandt *et al.*, 1963). In the later phase of oral processing, the melting of gelatin would make the emulsion-filled gel become liquid and lose its structure. Thus, flow properties of the molten samples would become more important, and probably even overruled the effect of the fracture properties (cf. gelatin gels studied by (Devezeaux de Lavergne, 2015) and discussion in section 7.4.2.2). In addition, the melting of gelatin also influenced the contribution of food particles to the perception of fat-related attributes. Thus, for future investigation, in the case of systems that exhibit melting behavior (such as gelatin types of desserts, ice creams, cheeses, and starch-containing desserts), it would also be important to study the friction behavior of molten gels or gels in a melting process, bearing more relevance to the late phase of oral processing. Preferably, it is necessary to couple lubrication measurements and other physical characterizations as close to the oral environment as possible. It was found in this thesis that

oral processing time and follow-up consumption has influence on fat fractions deposited on the tongue and on fat perception. Different chewing behaviors were reported to influence the texture perceptions of solid foods. Thus mimicking oral processes also requires taking of physiological aspects on an individual and group level to understand and predict sensory perception at various stages of oral processing.

Increasing SFC was found to enhance fat coalescence during the breakdown of emulsion-filled gels, but this did not influence the perception of fat-related attributes. This was ascribed to the decreased difference of SFC at oral temperature. It is not known how much difference in SFC would cause noticeable difference in perception under oral conditions and which level of SFC is sufficient to cause more coalescence under oral conditions. One should be aware that fat particles with too high SFC might be perceived as gritty (or waxy if partially coalesced). A more effective design would require certain types or blends of fat containing just the right amount of solid fat, high enough at oral condition to induce coalescence of fat droplets, and not so high that it causes unpleasant perceptions. Inspiration might be obtained from the existing understanding on the melting of fat in chocolate, where the melting temperature of fat is precisely controlled.

The differences of mechanical/fracture properties of all the gel systems in this thesis are relatively small, and further reduced under the influence of the melting of gelatin. In these cases, the differences in lubrication properties induced by different structures overruled the effect of mechanical properties on textural perceptions. We note that the differences in mechanical properties of foods are in reality much larger than the differences between the systems treated in this thesis. Therefore, the findings of thesis are most directly applicable to semi-solid foods, with soft textures. For obtaining a better understanding of how mechanical and lubrication properties are interrelated and how these properties influence sensory perception, other types of gel matrices with a larger variation in mechanical/fracture properties should be studied. In addition, the liquid systems studied in this thesis were thin Newtonian fluids, while in reality, liquid foods such as drinking yoghurts can be thicker and shear thinning, thus a larger variation of viscosities of liquid systems should be considered as well.

Tween 20 was used in this thesis to create droplets that are unbound to the matrix. The disadvantage of using Tween 20 is that it gives a bitter taste, which might have a negative effect on product quality and liking, and the perception of other attributes such as creaminess. In addition, Tween 20 was found to have a strong effect on lubrication. This reduced the effect of fat droplets themselves on lubrication and perception. It is advised to

seek different types of food grade emulsifiers that can regulate the binding properties of fat droplets in gels, while not affecting the taste and lubrication properties.

This thesis tested a limited range of particle sizes (fat droplets and small MWP particles versus large MWP particles) and shapes (spherical fat droplets and protein particles versus polyhedral starch particles). To further understand the impact of food particle properties on the rheological, tribological and sensory properties of particle-containing foods, particles types with larger ranges in morphology, size, and deformability should be tested. Considering the hydrophobic nature of fat, as well as its ability to deliver flavors, particle properties such as hydrophobicity and encapsulation capability should also be considered.

### 7.5.2 Implications of current findings

In the beginning of this chapter, a schematic overview of the approach of this thesis was presented (Figure VII - 1). The outcomes of this thesis were incorporated in the structure of this figure, yielding an integrated model for food design (Figure VII - 11) that highlights the role of structural elements (esp. food particles) in determining the structure, rheological and tribological properties and sensory perception of foods.

This integrated model contributes to improving the effectiveness and efficiency of food product development. This model indicates the importance of particle properties in both the lubrication properties and sensory perceptions of foods, and emphasizes the different lubrication mechanisms of different structure elements in relation to perception. This model also highlights the differences in effects of food particles in liquid versus semi-solid gel systems. These findings provide an instructive guideline in the selection and application of suitable food structural elements for specific food products. In addition to this, these findings suggest criteria for developing other ingredients as possible fat replacers, and allow the prediction of their texture and sensory perceptions. Furthermore, the discrepancies between the results obtained from physical measurements and sensory tests point out the importance of considerations of more oral-imitative conditions. These considerations would help future researchers to obtain more relevant and applicable results.

The knowledge obtained in this thesis is especially important for designing reduced-fat products that contribute to a healthy diet. Several fat reduction and replacement strategies are suggested, including increasing the availability of fat, improving the lubrication by particle ball bearing, and reducing the perception of negative attributes, such as roughness. Products with improved lubrication properties are also applicable for target groups, such as people with dysphagia or a dry mouth.

## 7.6 Concluding remarks

Food structural elements can be manipulated to control the tribological properties of food model systems. Morphology, size, and deformability of food particles determine the lubrication behavior of the food systems. Spherical particles with micrometer size are able to reduce friction through a ball bearing mechanism, while irregularly shaped particles increase friction by increasing the apparent surface asperity contacts. Deformable particles can flatten the surface by filling asperities, thus reduces friction. Coalescence of unstable droplets can plate-out on the surface and form film patches, thus reduces friction. Other structural elements, such as emulsifiers and sticky molecules, also influence tribological properties of the systems.

Interactions between the food structural elements can influence the rheological properties of liquid food systems and the fracture properties of semi-solid food systems. These properties as well as the tribological properties are interrelated and all of them affect sensory perception. The interrelations between physical and sensory properties of food systems are different for liquid and semi-solid systems. On one hand, semi-solid systems limit the availability of food elements during consumption that contribute positively to desired sensory perception, thus structure design to improve the availability of these elements is of great importance for semi-solid systems. On the other hand, semi-solid systems mask the perception of unwanted attributes from large particles, such as roughness, which is perceived as dominant in liquid systems.

As depicted in Figure VII - 11, the relationships identified in this thesis provide generally applicable mechanisms to control and engineer the sensory properties of both liquid and semi-solid food systems by manipulating the food structural elements. The knowledge obtained in this work delivers valuable information for food researchers and industry to design or modify food products with targeted texture and sensory properties.

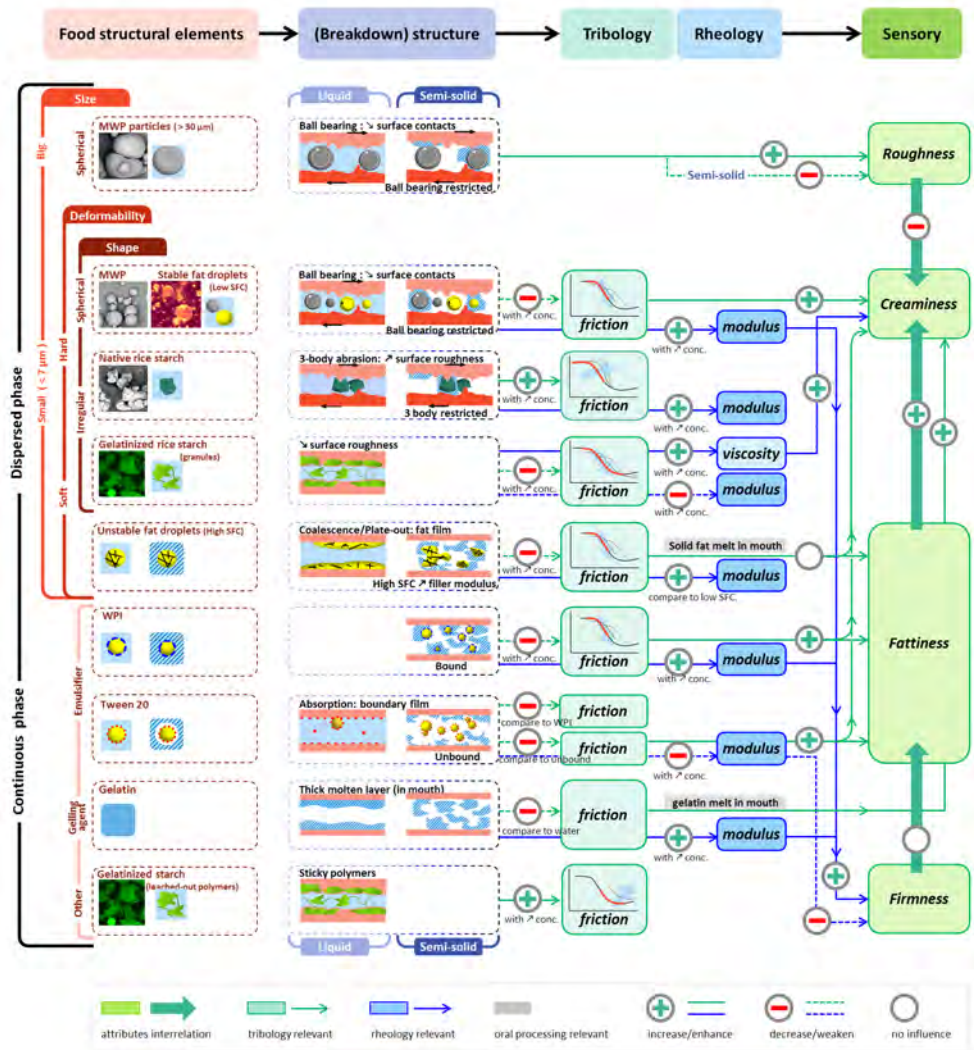


Figure VII - 11 An integrated model for food design: manipulating food structural elements and physical properties to achieve targeted sensory perception.





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# Summary

## Summary

Food products need to meet several requirements for nutrition, texture, sensory expectation and perception. Understanding the relationship between food structure and texture perception can help improve the design of food products tailored to specific consumer preferences. The structure of food is determined by its composition and the interaction between the compositional or structural elements. Both food structure and the texture perception of foods undergo dynamic changes during different phases of oral processing. During oral processing, both rheological and tribological properties of foods are relevant for sensory perception. Tribology, being the study of friction, lubrication and wear, is emerging as one of the key tools in understanding oral processing and mouthfeel. The general aim of this thesis was to understand the relationship between the structural properties, rheological and tribological properties during food breakdown, and the sensory perception of foods. More specifically, this thesis aimed to link the properties of food particles in liquid and semi-solid matrices to the tribological and rheological properties, and in this way, understand the sensory perception of these systems. This thesis also aimed to unravel the underlying fat mimicking mechanisms of particle fat replacers. Fat droplets and particle fat replacers based on protein and starch with micrometer size were investigated. These particles were dispersed in liquid and semi-solid gel phases, forming the food model systems under consideration.

**Chapter 1** gave the background information of rheology and tribology in food research. A brief overview of the available literature on the relationships between physical properties and perception of food texture, and on particle fat replacers was given. The aim and outline of the thesis were presented towards the end of this chapter.

**Chapter 2** investigated the effect of fat droplet characteristics in emulsion-filled gels on their dynamic rheological, tribological and microstructure properties during breakdown, as well as their sensory perceptions. The fat droplet characteristics were varied by modulating the interaction of the fat droplets with the gelatin gel matrix (i.e. bound or unbound to matrix), changing the fat droplet concentration and solid fat content (SFC) in the droplets. A mouth-mimicking tribometer connected to CLSM was used to determine the friction and microstructural evolution of gels under shear. It was found that fracture properties of emulsion-filled gels were affected by droplet-matrix interaction, droplet concentration and solid fat content. Gels with unbound droplets led to more coalescence of the fat droplets than bound ones and higher droplet concentration also led to more coalescence. The observed increase in fat coalescence was related to a decrease in friction, which was in turn related to an enhancement of the fat-related perception determined by quantitative

descriptive sensory analysis (QDA). The effects of unbound droplets and higher fat droplet concentration on increasing coalescence and decreasing friction were further enhanced by increasing SFC. However, increasing SFC did not further enhance the fat-related sensory perception. This was attributed to the melting of SFC in mouth and the complicated breakdown behavior of gels compared to simple emulsions studied in literature.

Fat droplet characteristics were shown in chapter 2 to affect the fat release and coalescence in a tribometer. **Chapter 3** further investigated the effect of fat droplet characteristics on the deposition of fat on the tongue in relation to sensory perception. The formation and clearance of fat fraction in oral coatings deposited on the tongue was determined by *in vivo* fluorescence. In addition, the influence of oral processing as well as follow-up consumption of liquid and semi-solid food gels on the clearance dynamics of fat deposition was also investigated. It was found that gels with unbound droplets or higher fat droplet concentration had higher fat fraction deposited on tongue, and higher fatty perception. These quantities also increased with increasing mastication time, and decreased after expectoration with increasing clearance time. Water was found to remove deposited fat from the tongue faster than semi-solid gelatin gel did. Studies about oral coatings that are formed after consumption of semi-solids and solids foods are scarce. This chapter shows the possibility of characterizing the fat fraction in oral coatings after consumption of semi-solid food gels, and it provides knowledge on the dynamic formation and clearance of fat deposition in oral coatings of food gels.

As consumer demand for healthier foods with lower amounts of fat is increasing, many microparticulated ingredients have been developed as potential fat replacers. Their fat mimicking mechanisms were previously not well understood. **Chapter 4** investigated the tribological and rheological properties of microparticulated whey protein (MWP) in liquid and semi-solid food model systems to reveal the mechanisms underlying the fat mimicking properties of MWP. MWP particles in liquids exhibited good lubrication properties. After scaling out the impact of viscosity on lubrication, this chapter provided strong evidence that the lubrication mechanism of MWP is mainly ball bearing. MWP in semi-solid gelatin gels also decreased friction, but to a smaller extent compared to the liquid model systems. For a multi-component semi-solid food gel, the mechanism underlying its lubrication behavior is complex due to the combined effects from the lubrication behavior of different components. Different from the ball-bearing lubrication of MWP particles, fat droplets (with high SFC) reduce friction due to coalescence and thus the formation of a fat film following a plate-out mechanism. Emulsifier Tween 20 influenced the friction by affecting the formation of the boundary emulsifier film as well as the fat film on the surfaces. It was suggested as a

next step to relate the tribological and rheological properties of the MWP particles to their sensory perception in different food matrices. This would allow developing and selecting suitable fat replacers for specific food products with the required textural and sensorial properties. The ball bearing lubrication of MWP particles revealed possibilities of using other food colloidal micro-particles as fat replacers.

As was recommended in chapter 4, the sensory properties of MWP particles in relation to their rheological and tribological properties were studied in **chapter 5**. Liquid MWP-o/w emulsions with controlled viscosities and semi-solid MWP-emulsion-filled gelatin gels were used as food model systems. Sensory (QDA) results revealed that small MWP particles (smaller than the detection threshold) contributed to perception of creaminess due to their lubrication characteristic. MWP particles that were bigger than the detection threshold contributed to the rough and powdery perception, and thus suppressed perception of creaminess. MWP particles did not contribute to perception of fattiness in contrast to oil droplets. The perception of fattiness was probably related to the film formation properties of oil. As a result, MWP in liquid emulsions was generally perceived as rough but not creamy. In the case of MWP-emulsion-filled gels, although the gel matrix restrained the lubrication function of MWP particles, it also masked the rough perception of big MWP particles. Due to the combined effect of both oil droplets and MWP particles, MWP in gels resulted in an overall positive effect on the creamy perception. The main conclusions of this chapter were that MWP contributed to fat-related sensations in a different way than oil did. The perception of MWP particles was related to the size of the particle as well as the properties of the surrounding matrix. Thus, it is of great importance to consider the effect of the food matrix when applying particle fat replacers in foods.

Different from the spherical shape of MWP, micro-granular rice starch, being another type of particle fat replacer, exhibit polyhedral shape (native uncooked starch) and ability to deform (gelatinized starch). Tribological properties of both native and gelatinized rice starch in liquid o/w emulsions and semi-solid emulsion-filled gels were investigated in **chapter 6**. Native rice starch particles behaved as active fillers and increased the gel modulus with increasing concentration. At low concentration, soft gelatinized rice starch particles decreased gel modulus. At high concentration, gelatinized rice starch increased the gel modulus due to dense packing of particles. Native and gelatinized rice starch increased friction coefficients with increasing concentration due to different mechanisms: native rice starch particles probably increased friction due to their irregular shape and their agglomeration (at high concentration), which indirectly increased surface roughness and asperity contacts, while gelatinized rice starch particles likely increased friction due to the

stickiness of leached-out starch polymers. The presence of oil droplets in the case of rice starch-o/w emulsions could reduce the friction caused by this stickiness. The tribological behaviors of rice starch-emulsion-filled gels were more complex than liquids due to the bulk properties and breakdown properties of the gel matrix. The main conclusion of this chapter was that morphology and surface properties of the starch particles, bulk and breakdown properties of matrices were the main factors that determined the tribological properties of food model systems containing rice starch particles. This conclusion gave an indication that the fat-mimicking functionality of rice starch was probably not due to their contribution to lubrication, but due to other factors, such as contributions to thickness and melting.

**Chapter 7** summarized the main findings of all the previous chapters and gave a general discussion about the underlying mechanisms of the tribological behaviors of different particle-filled food systems. In general, food structural elements could be manipulated to control the tribological properties of food model systems. Interactions between the food structural elements could influence the rheological properties of liquid and semi-solid food systems. These properties as well as tribological properties were inter-related and all of them affect sensory perception. The inter-relations between physical and sensory properties of food systems were influenced by oral processing, such as oral processing duration and temperature. Several fat reduction and replacement strategies were suggested, including increasing the availability of fat that is in contact with oral surfaces, improving the lubrication by ball bearing of particles, and reducing perception of negative attributes such as roughness. Based on the main findings obtained from previous chapters, an integrated model was presented. This model indicated the importance of food particle properties in both the tribological properties and sensory perception of foods, and emphasized the different lubrication mechanisms of different structure elements and their relation to perception. This model also highlighted the differences in behavior of food particles between liquid and semi-solid gel systems.

In conclusion, this thesis contributed to a better understanding of the tribological properties of food particles in different food matrices on their sensory perception, as well as the relationship between food structure, tribological and rheological properties and their sensory perception. The knowledge obtained from this thesis should allow food researchers and industry to design or modify food products with targeted texture and sensory perception, and it is especially important for designing reduced-fat products that contribute to a healthy diet.





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In less than one minute you may have already flipped through all the pages of this book, the completion of which cost me four years of hard work and attention. You may have seen the nicely aligned paragraphs and elaborately designed figures, which were derived from chaotic draft versions and numerous revisions. I want to give myself a big applause, for having the necessary curiosity and initiative, for being meticulous and critical, and most importantly, for believing in myself, being perseverant and strong. Yes, I succeeded, with pride and pleasure, with joy and tears!

This thesis yet still owes its existence to the significant contributions of many others. My most sincere gratitude goes to my supervisors. They gave me solid guidance while also enough freedom to experiment. One simply could not wish for better supervisors. Dr **Fred van de Velde** provided me with so many insightful ideas and constructive suggestions. He helped me build up the comprehensive framework of my thesis, and many times dragged me away from unrealistic ambitions. He is very influential to me: since I started working with him, I have become more critical and rigorous in my research, and I have developed a planned and orderly way of working. His input was essential to the development of my thesis. I was always impressed by his vast knowledge and solutions, and I always felt “safe” and confident in my work having him as my supervisor.

Dr **Markus Stieger**, who offered me the opportunity of doing this job, gave me so much support and encouragement. Without him, I would never have the chance of presenting my work, learning and networking all over the world. His patience in guiding me through all kinds of problems, together with his optimistic and reassuring attitude, is particularly vital for my achievements. His expertise in sensory science has contributed a lot to the design and execution of my sensory studies, which for me were the most difficult parts of this work. Besides, I always had the feeling that my manuscripts rose from the ashes like a phoenix every time after his corrections.

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Of course, my achievement of the Doctor’s title would not happen without the evaluation

from my thesis committee members. I would like to thank Prof. Dr **Tiny van Boekel**, Dr **Anwasha Sarkar**, Dr **Chrystel Loret**, and Prof. Dr **Jozef Kokini**, for their precious time reading my thesis, for their appreciation of my humble work, and for their effort in traveling far to my defense.

It might be confusing: my PhD project belonged to a TI Food and Nutrition (TIFN) project with Vion Food Group and FrieslandCampina as project partners. I was bound to the Food Physics Group in Wageningen University but I was mostly located at NIZO food research during the first two years. My special thanks go to **Ronald Klont**, **Hans Hage**, **Marcel Paques**, **Yves Nicolas**, and **Mirre Viskaal** for providing me with enough research materials and giving me positive feedback on my presentations. I enjoyed every expert meeting with their involvement and I had great experiences visiting FrieslandCampina in Amersfoort and Wageningen.

The start of my PhD would not have gone so smoothly without my dear team members **Sara Camacho**, **Marine Devezeaux**, and **Judith Derks**, the three other PhDs in the project. Soon we became very close friends. We have so many nice memories together, in our old office in Biotechnion, in the conferences in Beaune, Amsterdam and Paris, in all kinds of courses, in weddings and parties, in the Efteling, and in the cinema. I owe them so many thanks for helping me with my first sensory study! A special thanks to Sara, for being such a nice colleague with whom to collaborate on both a project and a paper. I learnt a lot from her straightforward and passionate way of working. I also owe her and **Sven** a big hug for bringing me back home when I was dead drunk!

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I will also never forget the companionship of my NIZO colleagues. **Jan Klok** was always inspiring and patient in helping me with my tribology, DSC, light scattering and CLSM experiments (together with many amusing stories); **Marijke Adamse** helped me with the homogenization and with finding things in the lab; **Saskia de Jong** helped me a lot with Instron; **Laura Oliver Hernandez** was a great source of ideas and she shared a lot of knowledge and lab tricks with me; **Ann Stijnmann**, **Celine Brattinga**, and **Jacqueline Voorendt** provided me with a lot of support in the FAC-Flex lab. And of course my dear friends who are/were at NIZO: **Inge**, **Adriana**, **Telma**, **Julia**, **Maria**, **Leila**, **Sarah**, **Carlo**, **Carlos**, **Caroline**, **Nina**, **Ines**, **Chao**, **Qu**, **Xiaowen**, and many others. I had so many cheerful moments, nice chats and events with them. Although many of them only stayed for half a year, our friendships last longer.

The wonderful time I spent in Food Physics was just unbelievable. I could not retrieve all my memories of these two years: so many activities and events. I want to express my gratitude to **Els Jansen**, who was always available to help me with all the trivial things and giving me administrative support. On average I ran to her office twice a day and always got satisfying answers. With her magic hand I could defend my thesis on this very relaxing and joyful Friday afternoon, not to mention how many different ways she tried to motivate me practicing my Dutch. There was my dearest friend **Anika Oppermann**, who is one of my paranympths by my side. We had so many beneficial discussions on research (sensory and tribology), and also many sympathetic conversations about life and love. We went through our friendship crisis together, learnt a lot from each other, and afterwards we both appreciated everything that we could share with each other. During my PhD, I spent most of my spare time activities with her. There was the girl who loved pink, **Jinfeng Peng**, being my closest buddy in the group, who always faithfully listened to me and gave me her best words. During the last three months of finishing up my thesis, I was often too busy to cook, and she always “accidentally” cooked more food to share with me. During the last three months of finishing up her own thesis, it was still she bringing me food with great generosity. Shouldn’t I feel so guilty and grateful? There was the superwoman **Elke Scholten**, who appreciated my “detailism”. She was my neighbor both at work and home, thus she always lent me her hand when I needed help. She is an excellent teacher and a great colleague. Outside work I had a lot of fun with her, drinking beer, gossiping, and making fun of Pingping. I also had so much nice time with my officemates, **Tijs** (who is always willing to help), **Auke** (who has a great sense of humor) and **Maria** (who always brings me positive energy), as well as my new officemates **Marco** and **Monica**. Although Food Physics was a small group, I was impressed by the many nice colleagues I had: **Harry**, **Miranda**, **Guido**, **Leonard**, **Paul**, **Zhili**, **Vaida**, **Anja**, **Claire**, **Carsten**, **Lenka**, **May**, **Pauline**, **Alev**, **Jacob**, **Min**, **Arianne**, **Carol**, **Leen**, **Belinda**, **Claudine**, and **Nam-Phuong**. I want to thank all of them for offering me so much support, especially during my

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*Kun*



# About the author



## Biography



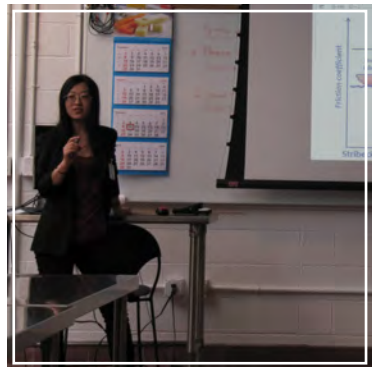
Fenjiu (汾酒)

Kun Liu was born on August 15, 1988 in Lüliang city, Shanxi Province, China. Her hometown is located along The Yellow River in the mid-North of China, where a lot of Jujube trees grow and from where the famous Chinese alcohol *fenjiu* originates.

After finishing her secondary education in her hometown, in September 2005, Kun started her Bachelor studies in *Beijing Forestry University*, where she majored in Food Science and Engineering. She completed her Bachelor thesis in the *Institute of Process Engineering, Chinese Academy of Sciences*, in Beijing. Her thesis was mainly focused on the purification and quantification of a healthy antioxidant component (SDG) from flax seeds. In August 2009, Kun took a big step to do her Master studies in *Wageningen University* in the Netherlands, where she started eating cheese and Dutch “drops”, and biking in the rain. Her Master study was specialized in Food Ingredients and Functionality, thus a lot of chemistry and physics were involved. Kun was interested in things that make “sense”, such as colors, tastes, and flavors. In 2010 she completed her Master thesis in the *Laboratory of Food Chemistry* in Wageningen, where she purified and quantified green and red colorants extracted from microalgae and turned them into blue colors. In 2011 she got the opportunity of doing her internship in a flavor and fragrance company, *Givaudan*, in Dübendorf, Switzerland. There, her task was to encapsulate water-insoluble flavors and flavor-blockers in water-soluble delivery systems. Thus she was able to make coffee less bitter and make chewing gum with long-lasting (24h+) cooling sensations. With her passion in sensory science and



her knowledge in food technology, she joined the TIFN project “Dynamics of texture and taste perception” in January 2012 as a PhD researcher in the *Laboratory of Physics and Physical Chemistry of Foods*, Wageningen University. She investigated how the properties of food particles and the microstructure of foods influence the friction during eating or similar conditions, and how this is related to sensory perception. During her PhD she supervised 6 MSc and BSc theses and attended many courses and conferences. Apart from doing science, she also participated in teaching practicals and organizing a PhD study tour in USA and Canada. In the last year of her PhD she obtained a Dutch driving license en ze kan sommige Nederlands spreken.



Kun likes painting (esp. flora and fauna) because she is good at colors and she loves nature. She likes reading detective books and science blogs, because she is always so curious about things. Now she just started her own blog about the science behind Chinese foods, hoping that she can contribute a bit to helping the world know more about her home country. Her favorite places to walk around are art galleries, zoos and botanical gardens, and she likes gazing at the stars.



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## List of publications

### Publications in peer-reviewed journals:

**Liu, K.;** Stieger, M.; van der Linden, E.; van de Velde, F. (2015). Fat droplet characteristics affect rheological, tribological and sensory properties of food gels. *Food Hydrocolloids* 44 (0): 244-259.

Camacho, S.<sup>1</sup>; **Liu, K.**<sup>1</sup>; Stieger, M.; van de Velde, F. (2015). Formation, Clearance and Mouthfeel Perception of Oral Coatings Formed by Emulsion-Filled Gels. *Journal of Texture Studies*. 46 (6): 399-410.

**Liu, K.;** Tian, Y.; Stieger, M.; van der Linden, E.; van de Velde, F. (2016). Evidence for ball-bearing mechanism of microparticulated whey protein as fat replacer in liquid and semi-solid multi-component model foods. *Food Hydrocolloids* 52: 403-414.

**Liu, K.;** Papadeis, P.; Stieger, M.; van der Linden, E.; van de Velde, F. (Submitted, 2016). Effect of microparticulated whey protein on sensory properties of liquid and semi-solid model foods.

**Liu, K.;** Stieger, M.; van der Linden, E.; van de Velde, F. (2016). Tribological properties of rice starch in liquid and semi-solid food model systems. *Food Hydrocolloids* 58: 184-193.

Weesepoel, Y.J.A.; Vincken, J.P.; Pop, R.M.; **Liu, K.;** Gruppen, H. (2013). Sodiatio as a tool for enhancing the diagnostic value of MALDI-TOF/TOF-MS spectra of complex astaxanthin ester mixtures from *Haematococcus pluvialis*. *Journal of Mass Spectrom* 48 (7): 862-874.

### Manuscript in preparation

**Liu, K.;** Stieger, M.; van der Linden, E.; van de Velde, F.. The role of tribology in understanding texture perception: a review of recent developments in tribology related to food

## Overview of completed training activities

### Discipline specific courses

- 2012, 1st Food Structure and Rheology, VLAG, Wageningen, the Netherlands
- 2013, 4th Advanced Food Analysis, VLAG, Wageningen, the Netherlands
- 2013, 14th European School on Rheology, KU Leuven, Leuven, Belgium
- 2013, Cambridge Tribology Course, University of Cambridge, Cambridge, United Kingdom
- 2013, 6th Sensory Perception and Food Preference, VLAG, Wageningen, the Netherlands
- 2014, Multivariate analysis for food data/sciences, VLAG, Wageningen, the Netherlands

### Conferences

- 2012, 2nd Food Oral Processing Conference, INRA, Beaune, France
- 2014, 3rd Food Oral Processing Conference, Wageningen, the Netherlands (Oral + Poster)
- 2014, 1st Food Structure & Functionality Forum Symposium, Amsterdam, the Netherlands (Oral)
- 2014, 15th Food Colloids Conference, Karlsruhe, Germany (Oral)
- 2014, 2nd International Conference on BioTribology, Toronto, Canada (Poster)
- 2015, 7th International Symposium on Food Rheology & Structure, ETHzürich, Switzerland (Oral)
- 2015, 12th International Congress on Engineering and Food, Quebec, Canada (Poster)
- 2015, 6th International Symposium Delivery of Functionality, Paris, France(Oral + Poster)
- 2016, 2nd Food Structure & Functionality Forum Symposium, Singapore (Oral)
- 2016, 16th Food Colloids Conference, Wageningen, the Netherlands (Oral)

### **General courses**

2012, VLAG PhD week, Baarlo, the Netherlands

2013, Scientific Writing, Wageningen in'to Languages, the Netherlands

2013, Voice and Presentation Skills Training, Wageningen, the Netherlands

2014, Techniques for writing and presenting, Wageningen, the Netherlands

2014, PhD Workshop Carousel, Wageningen, the Netherlands

2015, Career Perspective, Wageningen, the Netherlands

### **Optional courses and activities**

2012, Instrumental Sensory Science (MSc course), Wageningen, the Netherlands

2012, Preparation of research proposal, Wageningen, the Netherlands

2014, Participation and organization of PhD study tour to USA and Canada

2015, TIFN course "how to get the right message across", TIFN, Ede, the Netherlands

2012-2016, Weekly group meetings, FPH, Wageningen, the Netherlands

2012-2016, TIFN project meetings + expert meetings + partner visits, the Netherlands



## Colophon

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# Propositions

1. Both ball bearing lubrication by small particles and plate-out lubrication by oil droplets contribute to perception of creaminess.  
(this thesis)
2. Fat reduction strategies should focus on retaining fat that is in direct contact with oral surfaces.  
(this thesis)
3. The work of Lake *et al.* that argues that probabilistic program induction enables human level concept learning implies that human intelligence in how to teach a machine is now the essence of further developing artificial intelligence to a human level.

Lake, B. M. *et al.* (2015) Human-level concept learning through probabilistic program induction. *Science*. 350:6266

4. The cause of the climatic modulation of mid-latitude cyclones as formulated by Wang *et al.* neglects global economics as an important factor.

Wang, Y. *et al.* (2014) Asian pollution climatically modulates mid-latitude cyclones following hierarchical modelling and observational analysis. *Nature communications*. 5:3098

5. From a sustainability point of view, governments should enforce punitive measures to combat wasting food.
6. Cultures that suppress complaining about problems are suffering from this suppression.

Propositions belong to the thesis entitled:

“Lubrication and perception of Foods:  
tribological, rheological and sensory properties of particle-filled food systems”

Kun Liu

Wageningen, 15 April 2016

# 食物的润滑与口感

颗粒填充型食品体系的摩擦和  
流变性质及感官性质

## 总结

本文属于博士论文《食物的润滑与口感：  
颗粒填充型食品体系的摩擦和流变性质及感官性质》

作者：刘坤  
二零一六年四月十五日



## 总结

食品需要满足人们对营养、质构以及感官体验等各个方面的需求。理解食物结构与其感官体验之间的关系有助于有针对性的根据消费者的特定需求来设计开发新的食品。食物的结构是由食物的组成成分，以及这些成分之间的相互关系来决定。食物的组成成分亦可称之为“结构元素”。在进食的过程中，食物的结构以及食物所赋予我们的感官体验均处于动态的变化之中。在咀嚼和吞咽过程中，食物被不断地研碎、与唾液混合、流动、变形，并与口腔、牙齿和舌头充分接触，因此研究食物在口腔中的流变与摩擦性质对于我们理解食物的感官性质是非常重要的。摩擦学是研究摩擦、润滑与磨损的学科。近些年来它在帮助人们理解口腔加工与食物感官性质方面起到关键作用。这本博士论文的主要目的即是理解食物在被咀嚼研碎的过程中其结构变化、流变与摩擦性质、感官性质之间的联系。更具体来说，这本论文意图理解食品中的颗粒是如何影响它们在液态及半固态的食品中的流变、摩擦以及感官性质。此外，本论文亦致力于揭示微颗粒化脂肪替代物模拟脂肪的机理。本论文中所研究的食物颗粒包括乳化的脂肪液滴、乳清蛋白颗粒以及淀粉团。这些颗粒都是微米级大小，并被均匀地分散在液态或半固态的溶胶体系中。这些体系即是本论文所采用的食物模型。

论文的**第一章**概述了流变学与摩擦学在食品科学研究中的背景知识。根据现有的文献资料，本章简要地介绍了食品质构与感官性质和其物理性能之间的联系，并简述了微颗粒化脂肪替代物的性质。本章的结尾叙述了论文的目的和大纲。

**第二章**研究了脂肪液滴特性对填充型半固态凝胶在研碎过程中的动态流变性质、摩擦性质、微观结构和感官性质的影响。在本章中我们调节了以下几种脂肪液滴的特性，包括：调节脂肪液滴与其所在的明胶基质之间的相互作用（即液滴与明胶基质或紧密结合，或松散结合）；调节脂肪液滴在明胶基质中的含量；调节脂肪液滴中固态脂肪的含量。我们使用了一台与共聚焦激光扫描显微镜所连接的可以模拟口腔运动的摩擦计，来测量食物在研碎过程中摩擦力与微观结构的变化。我们发现，不同结构的食品乳液凝胶的破碎方式不同，而这些不同则是由液滴与明胶基质的相互作用、液滴在明胶中的含量、液滴中固态脂肪的含量所决定的。若凝胶中的液滴与基质松散结合，或增加液滴在基质中的含量，（比起与基质紧密结合的液滴，或较少量的液滴来说，）液滴更容易从基质中释放出来，并更容易与其他液滴相互融合。液滴的释放与其融合被证明与摩擦力的减少有关，并可增强在感官品尝测试（量化描述测试）中与脂肪相关的口感（例如奶油般的绵密滑腻）。若提高脂肪液滴中固态脂肪的含量，则松散结合的液滴或多量的液滴对液滴释放、融合、减少摩擦力的作用会进一步增强。然而，增加液滴中固态脂肪的含量并未对口感产生显著影响。这可以被归因于固态脂肪在口腔中的迅速融化（因此含量降低），以及半固态凝胶在口腔中复杂的研碎过程。这样的复杂研碎并不存在于文献中更常报导的相对简单的液态乳液体系中。

在第二章中我们提到脂肪液滴的特性会影响其在摩擦计中从凝胶体系中的释放与融合。而**第三章**则更进一步研究了脂肪液滴特性对其在口腔（舌面）上余留程度以及其口感的影响。（在进食过程中及之后食物会在口腔中（例如舌面）形成一层覆盖物，这层覆盖物会影响进食者对食物的感

官体验。）本章使用了荧光计来检测脂肪在舌面覆盖层中的形成与清除过程。此外本章也研究了口腔咀嚼过程以及后续进食（包括液态与半固态食物）对舌面覆盖层中的脂肪的清除效果的影响。我们发现，凝胶中若含有与基质松散结合的脂肪液滴或是高浓度的脂肪液滴，其在舌面覆盖物中脂肪余留的含量会升高，并提高油腻口感。此外，脂肪在舌面的余留以及油腻口感会随着咀嚼时间的延长而增加，并随着吞咽（或吐出）后时间的增加而减少。后续进食时，相较于进食半固态明胶，饮用液态的水可以更快的清除舌面覆盖层中的脂肪。关于进食半固态或固态食品后舌面食物覆盖层的文献报导非常之少。简言之，本章提供了一种有效的方法来测定进食半固态食物后口腔覆盖层中脂肪的含量。此外，本章也为研究口腔覆盖层中脂肪层的动态形成与清除机理提供了更多信息。

随着消费者对低脂健康食品需求的不断增长，很多微颗粒化的食品原料被应用（发展）为潜在的脂肪替代物。但是这些微颗粒化的原料模拟脂肪的机理尚未完全阐明。论文的**第四章**探讨了微颗粒化乳清蛋白在液态与半固态食品体系中的摩擦与流变性质，由此来阐明其模拟脂肪液滴的机理。研究发现，微颗粒化乳清蛋白在液态食品系统中展现了良好的润滑效果。在排除了粘度对其润滑效果的影响后，本章有力的证明了微颗粒化乳清蛋白的润滑作用主要是由于“滚珠效应”（请联想自行车中的滚珠轴承）。在半固态的明胶体系中，微颗粒化乳清蛋白亦起到润滑作用，但相较于其在液态体系中的润滑作用，程度较轻。当微颗粒化乳清蛋白与其他颗粒或组分共存于半固态食物体系中时，这个体系整体的润滑效果与机理则变得十分复杂，因为不同的组分对润滑（或摩擦）的影响及机理皆不相同。与微颗粒化乳清蛋白的“滚珠效应”不同的是，脂肪液滴（高固态脂肪含量）减少摩擦的机理是“平铺效应”，即脂肪液滴相互融合而在表面形成一层脂肪膜。这层脂肪膜因可以有效减少表面之间的直接接触而减少摩擦。此外，食品体系中的乳化剂，例如本论文中使用的吐温20也可以减少摩擦，其机理则为小分子的乳化剂吸附在表面形成小分子膜。乳化剂亦可协助脂肪液滴更有效地在表面形成脂肪膜（促进“平铺效应”）。本章建议进一步研究微颗粒化乳清蛋白在不同体系中的摩擦和流变性质与其口感之间的联系。这将允许我们针对性地根据特定的食品体系来研发和选择合适的脂肪替代品，从而满足人们对食品的质构和口感的需求。微颗粒化乳清蛋白的“滚珠效应”也揭示了使用其它球状微颗粒化食品原料作为脂肪替代品的可能性。

基于前一章的建议，**第五章**研究了微颗粒化乳清蛋白的感官性质以及其感官性质与其摩擦和流变性质的关系。本章使用的食品体系包括了统一粘度的液态体系以及半固态的乳液凝胶体系。这些体系均含有微颗粒化乳清蛋白。感官品尝测试（量化描述测试）指出，极小的乳清蛋白颗粒（小于舌头的品尝阈值，一般认为是约20微米）可以提高滑腻口感基于其润滑性能。然而，若乳清蛋白颗粒的大小大于舌头的品尝阈值，尽管仍有润滑性能，由于其过大的颗粒而产生的粗糙和颗粒口感，反而会抑制滑腻口感。此外，微颗粒化乳清蛋白并不能够提供脂肪液滴所提供的油腻口感。这很有可能是因为油腻口感和脂肪能够形成油膜这一特性有关。因此，我们发现微颗粒化乳清蛋白在液态体系中的总体口感是粗糙感和颗粒感，而不是滑腻。若微颗粒化乳清蛋白存在于半固态的凝胶体系中，尽管明胶基质在一定程度上限制了微颗粒化乳清蛋白的滚珠润滑效果，它也有有效的掩盖了

粗糙感和颗粒感。由于粗糙感的消失，以及脂肪液滴的协同效果，微颗粒化乳清蛋白在半固态体系中的总体口感便为滑腻的优良口感。本章的主要结论即为，微颗粒化乳清蛋白与脂肪液滴提供脂肪相关口感的机理不同，且微颗粒化乳清蛋白的感官性质与其颗粒大小以及所在的体系性质有关。因此，将微颗粒化食品原料应用于食品体系中时，考虑体系性质对这些颗粒的性质的影响尤为重要。

在**第六章**中我们研究了另外一种脂肪替代物：从大米中提取的微淀粉团。与球状的微颗粒化乳清蛋白不同的是，生的微淀粉团的形状为多面体。此外，加热糊化后的微淀粉团吸水变得柔软，因此极易发生形变。本章研究了生的微淀粉团与糊化的微淀粉团在液态溶胶与半固态凝胶体系中的摩擦性质。生的微淀粉团与明胶基质紧密结合，因此随着生微淀粉团浓度的增加，填充凝胶的弹性模量也会增加。柔软的糊化微淀粉团在低浓度时则会减小填充凝胶的弹性模量，但在高浓度时由于密实堆积则会增加填充凝胶的弹性模量。生微淀粉团与糊化微淀粉团均会随着含量的升高而增加食品体系的摩擦力，但二者机理不同。生微淀粉团增加摩擦力主要是由于其不规则的形状以及其在高浓度时的聚集，从而间接地增加了表面的粗糙度和接触面积。而糊化微淀粉团增加摩擦力有可能是因为在糊化过程中从淀粉团中游离出的直链和支链淀粉分子，这些淀粉分子有较大的黏性从而增加表面之间的摩擦。若体系中存在有脂肪液滴，由于脂肪液滴可以在表面形成油膜从而减少了由于黏性产生的摩擦力。若将微淀粉团应用于半固态凝胶体系中，由于半固态的研碎性质比液态的更为复杂，摩擦性质也会变得十分复杂。总的来说，本章指出，含有微淀粉团的食物体系的摩擦性质主要是由微淀粉团的形态与表面性质，以及体系基质的研碎性质来决定的。这个结论也表明了微淀粉团模拟脂肪液滴的机理很有可能不是润滑作用，而是其他因素，例如增稠或融化。

**第七章**概括总结了之前章节的主要研究结果，并着重讨论了不同的颗粒填充型凝胶体系的摩擦性质与机理。总的来说，我们可以通过改变食品的分结构来调控食品体系的摩擦性质。并且，食物组分之间的相互作用也可以影响液态和半固态的食物体系的流变性质。食物的流变与摩擦性质是相互关联的，且可以进一步影响食物的感官性质。口腔加工过程，例如口腔咀嚼的时长以及口腔温度，会进一步影响食物的物理性质与感官性质之间的复杂联系。本论文建议了几种减少或替代食品中脂肪含量的策略，例如设法提高脂肪液滴与口腔表面的直接接触，或通过利用球状微颗粒的“滚珠效应”来提高食品的润滑效果，亦或直接减少不良口感（例如粗糙）从而减少其对良好口感的负面抑制。基于之前章节的主要结论，本章给出了一个综合模型。这个模型显示了食物中的颗粒对其物理和感官性质的重要性，并着重强调了不同颗粒在食品中的摩擦性质和感官性质的不同及机理。这个模型亦强调了食品颗粒在不同体系（即液态与半固态）中性质的不同。

综上所述，这本论文对更好的理解食物颗粒在不同体系中的摩擦和流变性质对食物感官性质的影响贡献了重要信息，并且有助于我们阐明食物结构、食物的摩擦及流变性质、食物的感官性质之间的联系。这本论文的研究成果将有助于食品研发者和食品企业设计或改进食品，以达到特定的质构和感官需求。这些成果将尤其有益于设计更加健康可口的低脂食品。