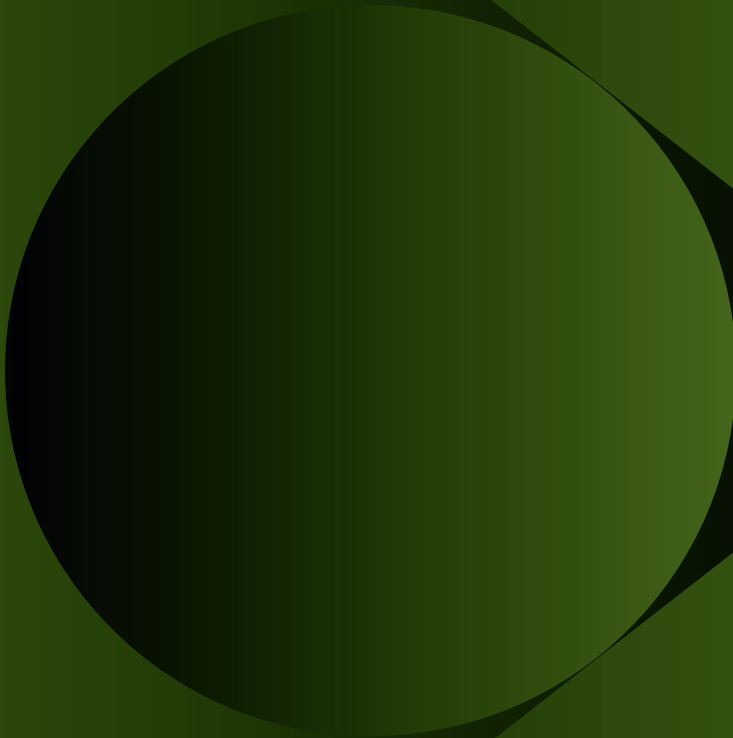# Clustering of oil droplets in foods

*Implications on rheology, tribology and sensory perception*

Philipp L. Fuhrmann

**Propositions**

1. Clustering of oil droplets is a more efficient fat reduction approach than replacement of fat by hydrocolloids. (this thesis)

2. Oil droplet cluster strength influences sensory perception more than cluster size. (this thesis)

3. Availability of a high diversity of crops to the consumer preserves genetic resources better than storing them at -80°C. (Tanksley, S. D., & McCouch, S. R. (1997). Seed Banks and Molecular Maps: Unlocking Genetic Potential from the Wild. Science, 277(5329), 1063 LP – 1066.)

4. Deactivation of mycotoxins in feed poses a higher risk to consumers than avoiding the occurrence of mycotoxins at farms. (Boudergue, C. et al.; EFSA Supporting Publications (2009))

5. We will feed the world through agriculture, not through the production of cultured meat. (Bekker, G. A., Fischer, A. R. H., Tobi, H., & van Trijp, H. C. M. (2017). Explicit and implicit attitude toward an emerging food technology: The case of cultured meat. Appetite, 108, 245–254.)

6. Globalisation is the primary driver for the growing incidence of food-related allergies.

7. Reducing friction is the most efficient way to limit climate change.

Propositions belonging to the thesis, entitled

Clustering of oil droplets in foods - Implications on rheology, tribology and sensory perception

Philipp L. Fuhrmann

Wageningen, 29 June 2020

# Clustering of oil droplets in foods

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# Table of content

# Chapter 1 - General introduction

## 1.1 Food structure design

Consumers increasingly demand healthier foods such as fat-reduced or plant-protein-based foods (Hathwar, Rai, Modi, & Narayan, 2012; Thiele & Weiss, 2003). However, changing the composition of foods while maintaining their sensory characteristics and consumer acceptability is still a challenge (Ares et al., 2017; Bayarri, Carbonell, Barrios, & Costell, 2011). The sensory characteristics of a food, consumer acceptance and food palatability are strongly influenced by food structure (Costell, Tárrega, & Bayarri, 2010; Guinard & Mazzucchelli, 1996). Engineering of food structure, therefore, might provide opportunities to tune the acceptability of food products by consumers. However, to use this strategy, a fundamental understanding of the interrelations between composition, structure, oral breakdown behaviour and sensory perception of foods is needed. In this thesis, these links will be explored. In particular, it will be investigated whether the structuring of the dispersed fat phase can be used to tune the mechanical properties and the sensory properties of foods. In this chapter, an introduction to food structure, rheology, tribology, sensory perception and their links is given. Subsequently, an outline of the thesis and its approach are provided.

## 1.2 Foods as multiphase materials

Many processed foods are multiphase materials, containing proteins, polysaccharides and fat. The ingredients and the interactions between the ingredients lead to specific microstructures and rheological properties, thus sensory characteristics (Dickinson, 2015; Sala, de Wijk, van de Velde, & van Aken, 2008; Stieger & van de Velde, 2013; van Aken, Vingerhoeds, & de Wijk, 2011; Szczesniak, 2002; Hutchings & Lillford, 1988). An ingredient with a significant influence on texture and sensory perception is fat. In foods, it is incorporated as droplets within a liquid or (semi-)solid matrix; examples are milk, yoghurt, sausages or cheese. Depending on their mechanical properties, many foods are either liquid oil-in-water (o/w) emulsions or (semi-)solid emulsion-filled gels. The effect of fat as a food ingredient on food structure, mechanical properties and sensory properties has been extensively investigated. It has been found that the properties of the dispersed fat phase, such as concentration, droplet size, droplet size distribution and droplet-droplet- as well as droplet-matrix interactions can directly affect the food properties and sensory perception. The effects of these properties on the mechanical properties and sensory perception will be further discussed in the next sections and are introduced schematically in Figure 1.
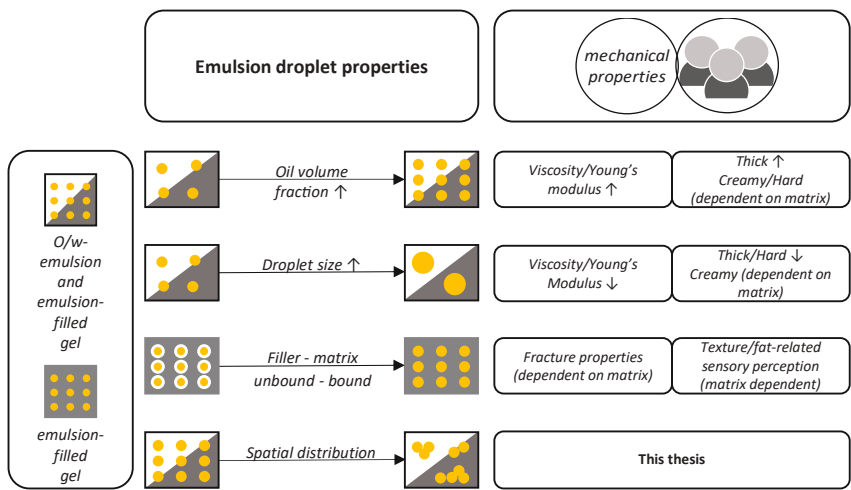
**Figure 1. Schematic depiction of the influence of emulsion droplet properties on mechanical properties and sensory perception. ↑refers to an increase, ↓ refers to a decrease. Half-filled squares refer to both emulsions and emulsion-filled gels, filled squares refer to emulsion-filled gels only.**

## 1.3 O/w emulsions and emulsion-filled gels

In many foods, fat is present as dispersed droplets of submicron- to µm-size (Capek, 2004). In stable emulsions, emulsifiers are adsorbed at the oil-water interface. They are substances or particles with an amphiphilic character. Upon adsorbing at the oil/water interface of the droplets, the emulsifiers stabilise the oil droplets by reducing or avoiding coalescence and flocculation. This stabilisation can occur, for example, through electrostatic repulsion, due to the presence of charged emulsifiers. If the emulsifying substances or particles are large enough, the oil droplets can also be stabilised by steric hindrance. In foods, the emulsifiers are typically small molecular weight surfactants (*e.g.* lecithin), proteins (*e.g.* whey proteins) or particles (*e.g.* microgels or starch granules (Linke & Drusch, 2018).

### 1.3.1 Rheology of emulsions and emulsion-filled gels

Emulsions and emulsion-filled gels can be characterised by their rheological properties. For liquid emulsions, the main parameter is the viscosity (Barnes, 1994). Emulsion-filled gels can be described by their moduli and fracture properties.

#### Rheological properties of oil-in-water emulsions

The flow properties of emulsions largely depend on the viscosity of the continuous phase, the absolute oil volume fraction of the oil droplets, the properties of the droplets and the interactions between the oil droplets. When the oil volume fraction is low (<0.01), emulsions show Newtonian flow behaviour. The viscosity, η, can be described by the Einstein relation as

$$\eta = \eta_c(1 + 2.5\varphi) \qquad\qquad 1$$

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in which ηc is the viscosity of the continuous phase, and φ is the volume fraction of the dispersed phase (Genovese, 2012). For the volume fraction range of 0.01 < $\varphi$ < 0.10, the Batchelor model (Eq. 2) can be used to describe the viscosity of emulsions and dispersions. It is a modification of Einstein accounting for hydrodynamic interactions (Jones, Leary, & Boger, 1991) .

$$\eta = \eta_c(1 + 2.5\varphi + 6.2\varphi^2)$$
2

In many foods, the oil volume fraction is higher. With increasing oil volume fraction, the emulsion exhibits a shear-dependent flow behaviour. For such emulsions, the viscosity can be reasonably well estimated using the Krieger-Dougherty model. In this model, the viscosity of the emulsion, η, depends on the viscosity of the continuous phase, ηc, and the volume fraction of the dispersed phase φ as

$$\eta = \eta_c * \left(1 - \frac{\varphi}{\varphi_{max}}\right)^{-[\eta]*\varphi_{max}}$$
3

As the dispersed droplets start crowding at higher oil volume fractions, and droplet-droplet interactions become relevant, a crowding factor $\frac{\varphi}{\varphi_{max}}$ is used. This crowding factor corrects for the divergence of the viscosity towards infinity, close to the maximum packing fraction, which is 0.64 for random close packing of hard spherical spheres. As oil droplets are deformable, the maximum packing of monodisperse droplets can increase to 0.74, if droplets pack hexagonally (Whitby, Lotte, & Lang, 2012). For the intrinsic viscosity, [η], a value of 2.5 is used for hard spheres.

The viscosity and flow behaviour of an emulsion also depends on droplet properties such as the droplet size, droplet stiffness, and size distribution. With smaller droplet sizes, the Laplace pressure increases, thus, the droplets become stiffer, and the viscosity increases (Pal, 1996). Also, for smaller droplet, the large interfacial area gives rise to more contact between the oil droplets, which leads to higher viscosities due to additional network formation. A polydisperse size distribution leads to a lower viscosity due to better packing (Otsubo & Prud'homme, 1994).

Mechanical properties of emulsion-filled gels

When the emulsion droplets are dispersed in a gel-like continuous phase, we refer to these materials as emulsions-filled gels. The mechanical properties of these gels are strongly affected not only by the matrix (*e.g.* type, concentration), but also by the emulsion droplet properties and the interaction of the droplet with the matrix (Figure 2A).
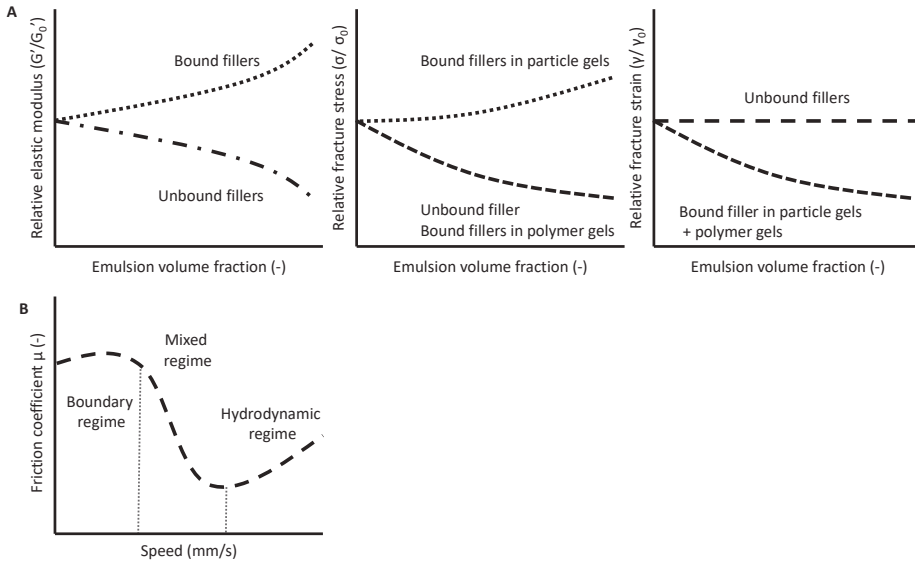
**Figure 2. (A) schematic depiction of the influence of emulsion droplet interactions and emulsion volume fraction on the elastic modulus and fracture stress and -strain. (B) Schematic depiction of a Stribeck curve.**

Two cases of droplet-matrix interaction can be differentiated; when droplets and matrix show attractive interactions, the oil droplets are bound to the matrix and referred to as active or bound fillers. For bound droplets, the effect of droplet incorporation into the gel matrix depends on whether the droplets are stiffer or less stiff than the continuous matrix. When the oil droplets are stiffer than the matrix, the elastic modulus of the emulsion-filled gel increases. Such an increase in droplet stiffness can be achieved by increasing the solid fat content (SFC) of dispersed fat droplets. When the SFC of the dispersed emulsion droplets increases, the elastic modulus of an emulsion-filled gel increases (Houzé, Cases, Colas, & Cayot, 2005; Mor-Rosenberg, Shoemaker, & Rosenberg, 2004; Mor, Shoemaker, & Rosenberg, 1999; Xiong, Aguilera & Kinsella, 1991). For liquid oil droplets, their stiffness depends on the droplet size. As the droplet stiffness is related to the Laplace pressure, which is determined by the surface tension and the droplet size of the droplets (van Vliet, 1988), decreasing the oil droplet size leads to an increase in gel stiffness (Kim, Renkema, & van Vliet, 2001; McClements, Monahan, & Kinsella, 1993). If the droplets are less stiff than the matrix, the overall gel stiffness decreases. Like in o/w emulsions, the oil volume fraction considerably changes the mechanical properties of an emulsion-filled gel; with increasing volume fraction, the reinforcement or softening effect is enhanced.

If the oil droplets do not interact with the matrix, droplets are unbound and referred to as inactive fillers; unbound oil droplets generally decrease the elastic modulus of a gel. Thus, droplet-matrix interactions influence the way that emulsion droplet properties affect the overall mechanical properties of an emulsion-filled gel.

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Besides the elastic properties, also the fracture behaviour, in specific fracture stress or fracture strain, characterises an emulsion-filled gel. The incorporation of emulsion droplets into a gel can result in either an in- or decrease of gel fracture stress, depending on the type of gel matrix (Sala, van Vliet, Cohen Stuart, van de Velde, & van Aken, 2009; Rosa, Sala, van Vliet, & van de Velde, 2006). With an increasing oil volume fraction in particle gels, *e.g.* WPI-based gels, fracture stress increases and fracture strain decreases. The increase of the oil volume fraction in emulsion-filled polymer gels, *e.g.* gelatine or kappa-carrageenan, decreases fracture stress and strain (Sala, van Vliet, Cohen Stuart, van de Velde, et al., 2009).

### 1.3.2 Lubrication properties of emulsions and emulsion-filled gels

As we consume foods, oral surfaces such as the teeth, the tongue and the palate are in contact with the food and with each other in a sliding motion. This motion can happen with a specific resistance, known as friction. If a high force is needed to move two surfaces against each other, the resistance or friction is high. Foods, in specific fat-containing foods, can coat the oral surfaces and reduce the force needed to slide them past each other. This means that foods can act as lubricants. The abilities of foods to lubricate oral surfaces and to reduce friction forces in the mouth are relevant to the fat-related sensory perception of a food item (Rudge, Scholten, & Dijksman, 2019; Sarkar, Ye, & Singh, 2017).

The lubrication behaviour can be described using the unitless friction coefficient μ and refers to the ratio between friction force ($F_F$) and normal force ($F_N$) as

$$\mu = \frac{F_F}{F_N}$$

4

The friction coefficient is a system property and depends on the presence and properties of a lubricant, on the surfaces and speeds at which it is determined. It is presented in a so-called Stribeck curve, where μ is shown as a function of the relative speed of two surfaces sliding against another. Three regimes are typically differentiated (Figure 2B). At low speeds, the friction coefficient remains constant with increasing speed; in this boundary regime, the two surfaces are in close contact. If the speed further increases, many lubricants cause a sudden drop in friction coefficient, which is known as the mixed regime. As the speed increases, small amounts of lubricant can enter the gap between the two surfaces, separate the surfaces, and therefore the friction forces decrease. At high speeds, the two entrained surfaces are entirely separated by the present lubricant, and hydrodynamic effects, as well as bulk properties, become relevant — the friction coefficient increases as a function of speed in what is called the hydrodynamic regime (Rudge et al., 2019).

The ability of a food item, in specific emulsion-type foods, to lubricate the oral surfaces is explained by two theories: the plate-out mechanism and ball bearing. These mechanisms and the influences of food structure and ingredients on lubrication are typically studied by using a tribological setup with surfaces proposed to mimic oral surfaces, in terms of *e.g.* mechanical properties (polydimethylsiloxane PDMS and PDMS or glass). From such studies, we have learned that the lubrication behaviour of o/w emulsions depends on parameters such as oil volume fraction, droplet stability, and emulsifier-type. The friction coefficient of an emulsion decreases with increasing oil volume fraction. The dispersed oil has a high affinity to the hydrophobic PDMS. With an increasing concentration of oil, the formation of an oil film on the PDMS layer, effectively separating the two surfaces, is easier. The ability of o/w emulsions to be "plated out" and to lubricate mouth-mimicking surfaces can be increased by droplet destabilisation and

the formation of oil patches. The formation of such oil pools can be enhanced by droplet coalescence and is therefore also related to the stability of the oil droplets. In the case of starch-stabilised droplets, the presence of salivary amylase will induce coalescence, which lubricates the tribo-pair PDMS/glass better than coalescence-resistant droplets (Dresselhuis, de Hoog, Cohen Stuart, Vingerhoeds, & van Aken, 2008). Oil droplets in o/w emulsions can also lubricate two surfaces by the ball bearing mechanism. In this case, the dispersed oil droplets remain intact and spherical between the surfaces. For emulsions lubricating by ball bearing, stiffer droplets will provide better lubrication than less stiff ones (Laguna, Farrell, Bryant, Morina, & Sarkar, 2017; Liu, Stieger, van der Linden, & van de Velde, 2015).

For emulsion-filled gels, much less research has been performed on the friction behaviour. However, also for such materials, influence of emulsion droplet properties has been found. Liu and co-workers showed that by increasing the solid fat content in emulsion-filled gels, the friction forces decrease. Chojnicka and co-workers pointed out that interaction between droplets and the gel matrix can influence the tribological behaviour. Unbound droplets, for example, can be easier released from the emulsion-filled gel and reduce the friction coefficient (Liu et al., 2015; Chojnicka, Sala, de Kruif, & van de Velde, 2009). Yet, many questions remain unanswered, including the effect of matrix properties on the friction behaviour or the presence of various other non-fat filler particles.

## 1.4 Sensory perception of emulsions and emulsion filled gels

### 1.4.1 Fat-related sensory perception

Fat contributes not only to the structure and mechanical properties of foods but also has a strong influence on the sensory properties and the palatability of foods (Drewnowski, 1997). The perception of fat is considered a multimodal sensation, which encompasses texture perception, but also smell and taste (Guichard, Galindo-Cuspinera, & Feron, 2018). As the perception of fat is very complex, it is captured in sensory studies using several attributes.

One of the central attributes of fat-related perception, specifically for emulsion- and emulsion-filled gels, is creaminess. There seems to be a consensus that the attribute creaminess describes a desirable characteristic in fat-containing foods, as it often shows links with high palatability (Weenen, Jellema, & de Wijk, 2005). Until now, it is disputed which sensory cues creaminess entails (de Wijk, Prinz, & de Wijk; Prinz, Jon, 2005; Szczesniak, 2002). However, there seems to be agreement that creaminess is a combination of a bulk contribution (rheology) and a surface contribution (tribology) (Dickinson, 2018). Other fat-related attributes include spreadability (Guichard et al., 2018), melting (Guichard et al., 2018), smoothness (Lett, 2016), coating (Oppermann, de Graaf, Scholten, Stieger, & Piqueras-Fiszman, 2017) and thickness perception (Oppermann, de Graaf, et al., 2017).

Many of these attributes are perceived while we eat, chew, and process foods in the mouth. Thus phenomena such as individual sensitivity to fat-related compounds, enzyme levels, salivary flow rate and others have been suggested to be of importance for fat-related sensory perception (Feron & Poette, 2013).

### 1.4.2 Food properties and the fat-related sensory perception

Fat-related sensory perception shows a considerable dependency on the food properties themselves. Le Calvé and co-workers reported that discrimination levels of fat content strongly differed, depending on

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the viscosity of the food (Le Calvé et al., 2015). In products with a high viscosity, differences in fat content are more difficult to perceive compared to low viscosity foods (Le Calvé et al., 2015). The sensory properties of foods are linked to their structure, rheological properties and molecular composition (Foegeding, 2007). To provide consumers with palatable foods, it is necessary to understand how these parameters relate to sensory perception.

For fat-containing foods, it has been found that the fat-related sensory perception is linked not only to the properties of the dispersed fat phase of foods, but also to bulk properties and the interactions between the dispersed phase and the bulk phase. Examples for these links between rheology and the sensory properties of foods are discussed in the following.

Fat-related sensory perception of o/w emulsions

The perception of fat-related and non-fat related sensory attributes of foods strongly depends on the mechanical properties and the microstructure of the foods (Dickinson, 2018). For o/w emulsions, rheological properties, such as the viscosity, considerably influence the sensory perception. Emulsions with higher viscosity are usually perceived as thicker and often creamier then emulsions with lower viscosity (de Wijk, Terpstra, Janssen, & Prinz, 2006). Such effects could be obtained by incorporation of thickening agents. Also, the oil droplet size of o/w emulsions can influence sensory perception, with smaller droplet sizes leading to higher creaminess perception (Lett, Yeomans, Norton, & Norton, 2016). Not surprisingly, by increasing the fat content of foods, an increase in fat-related sensory properties can be achieved. This increase in fat-related properties might be related to either an increase in viscosity or the presence of the fat itself, thus, the lubrication properties of the food. Recent research has shown the importance of the ability of oil in water emulsions to lubricate oral surfaces (Zhu, Bhandari, & Prakash, 2019; Laguna, Farrell, Bryant, Morina, & Sarkar, 2017; Carvalho-da-Silva, van Damme, Taylor, Hort, & Wolf, 2013; Selway & Stokes, 2013; Stokes, Boehm, & Baier, 2013). The lubricity of emulsions links to fat-related attributes such as creaminess. For equi-viscous emulsions with the same oil volume fraction, but different tribological properties, higher lubricity can lead to higher ratings of creaminess compared to emulsions with lower lubricity (Laguna et al., 2017).

Besides rheological and tribological properties of emulsions, also the dynamic alteration of the structure of emulsions during oral processing can affect fat-related sensory perception. Emulsion droplets can coalesce (Silletti, Vingerhoeds, Norde, & van Aken, 2007) or flocculate (Vingerhoeds, Silletti, de Groot, Schipper, & van Aken, 2009), particularly in the presence of saliva and under oral shear. These phenomena can change the fat-related sensory perception of such emulsions remarkably. By using an amylase-sensitive emulsifier, such as octenyl succinic anhydride (OSA)-modified starch, emulsion droplets can be destabilised during oral processing and improve fat-related sensory perception, compared to coalescence-resistant emulsions (Benjamins, Vingerhoeds, Zoet, de Hoog, & van Aken, 2009). On the other hand, in emulsions stabilised with positively-charged emulsifiers such as lysozyme, oil droplets can flocculate in the mouth due to electrostatic interactions with negatively-charged mucins, present in saliva; the flocculation and the removal of mucin can lead to a dry or rough sensation (Vingerhoeds et al., 2009). Finally, also the in-mouth melting of fats in o/w-emulsions can contribute positively to the fat-related sensory perception, with specific melting behaviours leading to more or less of a cooling-melting sensation (Galindo-Cuspinera, Valença de Sousa, & Knoop, 2017).

Fat-related sensory perception of emulsion-filled gels

For emulsion-filled gels, linking structure and sensory perception is more difficult than for liquid o/w emulsions. Overall food properties can often not be separated from the properties of the dispersed fat droplets. Also, food matrices can vary substantially in their properties, *e.g.* their melting behaviour or water holding capacity. Therefore, conflicting findings exist in literature concerning the linking of mechanical properties and the fat-related sensory perception of gelled foods. The oil volume fraction (Kim, Gohtani, & Yamano, 1996), droplet size (de Wijk et al., 2006) and fat composition (Liu et al., 2015) influence the fat-related perception of gels. For example, the creaminess of an emulsion-filled gel was found to increase with increasing oil volume fraction (Sala et al., 2008) and for samples with smaller droplets (de Wijk et al., 2006). However, other research described for larger droplets increasing creaminess (Kilcast & Clegg, 2002). Also, interactions between the droplets and the matrix can influence the fat-related sensory perception. Chojnicka and co-workers (2009) showed in emulsion-filled gels with unbound oil droplets that the droplets are released more easily from the matrix upon oral processing. The released oil droplets increased fat-related attributes, *e.g.* fattiness and creaminess, more than bound droplets (Chojnicka et al., 2009). On the opposite, Devezeaux de Lavergne and co-workers (2015) found no effect of the droplet-matrix interaction on the fat-related sensory perception of emulsion-filled gels (Devezeaux de Lavergne, van Delft, van de Velde, van Boekel, & Stieger, 2015). Also, the mechanical properties of the matrix by themselves can influence the fat-related sensory perception. Emulsion-filled gels with higher fracture strain, for example, were found to show higher creaminess (Devezeaux de Lavergne et al., 2015), while other research shows lower fat-related ratings (creamy) for gels with higher fracture strain (Liu et al., 2015). Thus, one should be careful in the generalisation of findings linking structure and sensory perception.

Like emulsions, the perception of emulsion-filled gels changes during consumption, as the structure of the gels changes. After being released from the food matrix, emulsion droplets can coalesce, flocculate or melt. For example, gels containing droplets that were released from the matrix during consumption and coalesced in the mouth were found to be perceived more creamy than gels with non-coalescing droplets (Liu et al., 2015). For gelled foods, (a) the ability of the matrix to melt at oral conditions and (b) structural changes in the food due to oral processing and bolus formation are linked to changes in the sensory perception. Melting of the matrix, for example, was linked to higher ratings of creaminess and coating perception (Sala et al., 2008).

In comparison to liquid o/w emulsions, less is known about the influence of the tribological behaviour of emulsion-filled gels on the fat-related sensory perception. It seems that the surface properties of the bolus in conjunction with oil release can affect tribological phenomena in mouth and the fat-related sensory perception. As discussed, for emulsion-filled gels with unbound oil droplets, an increase in perceived creaminess was found compared to gels with bound droplets. This might be related to better lubricity of the bolus compared to boli from gels with bound droplets. Also, gels with lower fracture stress were found to show high creaminess compared to gels with high fracture stress, possibly linked to lower oral friction due to the formation of soft gel fragments compared to hard fragments (Devezeaux de Lavergne et al., 2016; Liu et al., 2015). To fully understand the links between the tribological behaviour of emulsion-filled gel and their sensory properties, further research is required.

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## 1.5 Spatial distribution of emulsion droplets – Effects on food structure, properties and sensory perception

A parameter that has received only limited attention is the effect of the spatial distribution of emulsion droplets in foods. In most studies, emulsion droplets were distributed homogeneously. The term homogeneous distribution of emulsion droplets refers to a situation, where the emulsion droplets are dispersed equally throughout a matrix. However, oil droplets can also be inhomogeneously distributed. This situation is also referred to as a heterogeneous distribution. In the case of a heterogeneous or inhomogeneous distribution, we find oil-enriched or oil-depleted areas in the food; in the fat-enriched areas, the droplets will occur in flocculates or clusters.

Research has shown that the clustering of dispersed oil droplets changes both the rheological properties of the material as well as its sensory properties. The clustering of the droplets entraps the continuous phase and hence increases the effective volume fraction. The increase in volume fraction gives rise to a higher viscosity and might change the sensory perception. Considering the work on multiple emulsions, a material with inhomogeneous droplet distribution, (Dickinson, 2015; Oppermann, Piqueras-Fiszman, de Graaf, Scholten, & Stieger, 2016; Oppermann, Verkaaik, Stieger, & Scholten, 2017), one might argue that clustering of the dispersed phase in an emulsion could have a positive effect on perception allowing for fat reduction.

In literature, several strategies are known to induce an inhomogeneous distribution of droplets. In o/w emulsions, clustering can be achieved by (weak) attractive interactions, such as (a) electrostatic attraction (de Figueiredo Furtado, Michelon, de Oliveira, & da Cunha, 2016; Mao & McClements, 2012a, b), (b) van der Waals interactions (Tadros, 1996), (c) depletion flocculation (Berli, Quemada, & Parker, 2002), (d) bridging interactions (Silletti et al., 2007; Dickinson & Galazka, 1991), or (e) strong, attractive interactions such as covalent bonds between droplets (Lee, Choi, & Moon, 2006). The common characteristic of these flocculation mechanisms is that the dispersed particles maintain their original size; thus, the droplets have not coalesced.

As a result of droplet clustering in o/w emulsions, the viscosity increases (Maier, Zeeb, & Weiss, 2014; Dickinson & Golding, 1997). With increasing shear forces, clustered emulsions display a shear-thinning flow profile, due to destruction of oil droplet clusters or alignment of droplet-clusters in the flow direction. The effects of clustering on sensory characteristics are not apparent.

In emulsion-filled gels, an inhomogeneous distribution of oil droplets can be realised by (a) the manipulation of the gelation kinetics of the gel phase, which leads to a local phase separation between the continuous matrix and the oil droplets, resulting into areas with higher and lower fat content (Oliver, Wieck, & Scholten, 2016) or (b) the introduction of emulsion-filled microgel particles (Shewan & Stokes, 2013).

Similarly to o/w emulsions, the spatial distribution of oil droplets is relevant for the mechanical properties of emulsion-filled gels; it was observed that clustering of oil droplets could lead to a reinforcement of the stiffness of emulsion-filled gel, with increases up to 4 times (Oliver, Berndsen, van Aken, & Scholten, 2015b). However, the effect on sensory perception has not been investigated. The sensory perception of

emulsion-filed gels with an inhomogeneous droplet distribution was tested in another study, where the inhomogeneity was created at large length scales. Mosca and co-workers created layered emulsion-filled gels with different oil content in the different layers and showed that an inhomogeneous distribution of oil leads to an increased fat-related sensory perception (Mosca, Rocha, Sala, van de Velde, & Stieger, 2012).

These results highlight that the microstructure of the food and specifically also the spatial distribution of emulsion droplets can have significant effects on structure, mechanical properties, such as rheology and fracture properties, lubrication behaviour, and sensory characteristics of foods. However, to this point, there is a lack of understanding of how the spatial distribution of emulsion droplets influences food properties. For example, it remains unclear how specific cluster properties such as (a) the extent of clustering, (b) the clustering strength and (c) the type of interactions between droplets affect food properties (Figure 3).

By developing approaches that allow to vary these properties controllably, it is possible to study and understand better how such structural modifications of the dispersed emulsion droplet phase in o/w emulsions and emulsion-filled gels affect mechanical properties and sensory perception of oil dispersed foods, keeping the dynamic changes of the structure due to oral processing into account.
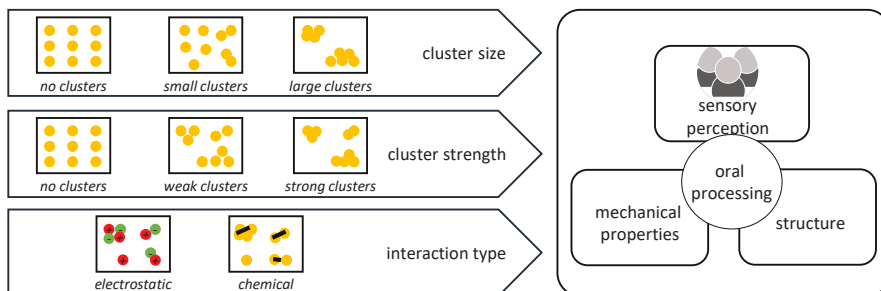


**Figure 3. Droplet clustering in o/w emulsions and emulsion-filled gels can affect mechanical properties and sensory perception.**

## 1.6 Outline and scope of the thesis

This project aimed to gain fundamental knowledge on the influence of the spatial distribution of emulsion droplets on mechanical properties, lubrication properties, and sensory perception of model emulsions and emulsion-filled gels. We aimed to gain an understanding of the mechanisms explaining the relation between the structure of the food and sensory perception, taking oral processing into account.

To obtain a defined spatial distribution of oil droplets, in this research, methodologies applicable to foods were developed that allow modifying the emulsion droplet distribution. By obtaining such methodologies, the effects of a specific oil droplet distribution and cluster properties on food properties and sensory perception can be studied. A schematic representation of the structure of this thesis can be found in Figure 4.

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Different approaches were tested that allow to control (a) the cluster size (and overall clustering length scale), (b) cluster strength and (c) cluster type. Methodologies to obtain a controlled oil droplet inhomogeneity in emulsions (**Chapter 2**) and emulsion-filled gels (**Chapters 4, 5**) were developed. By doing so, it was aimed to understand the effects of the spatial distribution and droplet-droplet interactions of oil droplets on the mechanical properties of liquid o/w emulsions and emulsion-filled gels (**Chapters 2-7**), as well as the relevant sensory properties (**Chapters 3-6**). In consideration of the difficulties of relating mechanical properties, structure and (fat-related) sensory perception, attempts were made to find correlations linking the changes in physicochemical properties to changes in sensory perception, taking changes in mechanical properties by oral processing into account. We did so by describing the rheology and tribology of emulsions before and after addition of saliva (**Chapters 3,5**) as well as the tribology of emulsion-filled gels (**Chapter 4**). Until now, limited studies describe the lubrication properties of emulsion-filled gels. In this thesis, we discuss a new approach to measure the friction behaviour of (soft) solid foods and food boli (**Chapter 8**). An exemplification of how clustering of oil droplets can be used as a fat-reduction strategy is provided for processed emulsion-filled protein gels (**Chapter 6**). In **Chapter 7**, we reiterate on the rheology of emulsions – extending the oil volume fraction to and above the critical oil volume fraction, to study the effect of clustering on yielding of emulsions.
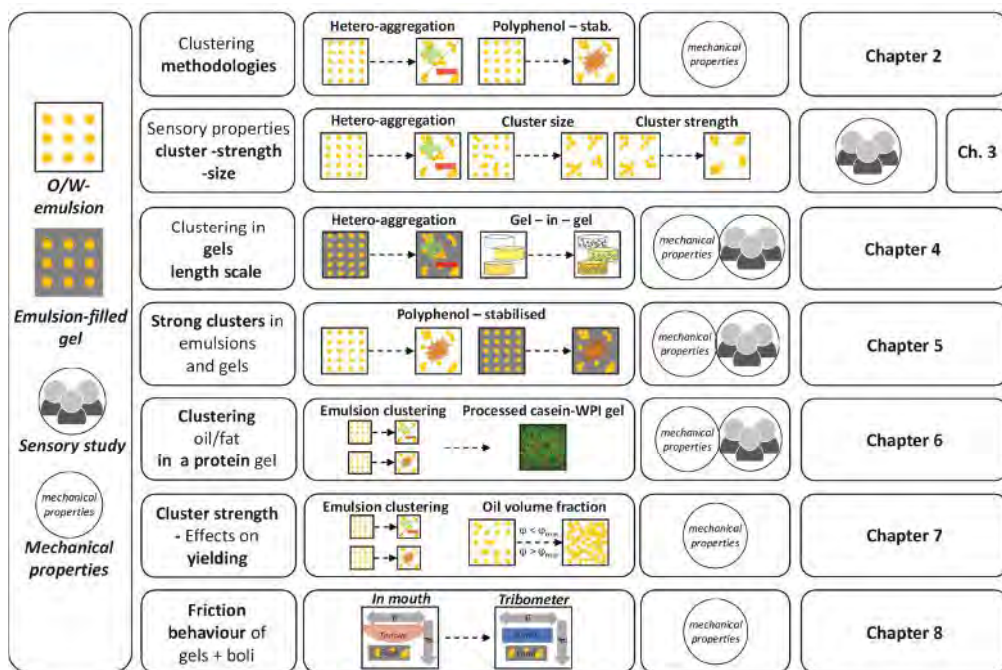


**Figure 4. Schematic depiction of the experimental chapters of this thesis**

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The output of this thesis can help to understand how to structure foods and similar materials, such as pharmaceuticals or cosmetics, by making use of the spatial distribution of oil droplets and droplet-droplet interactions. This might also allow reducing the overall fat content in specific food categories while maintaining desired fat-related sensory properties.

## 1.7 Approach

In this thesis, approaches to control the oil droplet distributions and the effects of the oil droplet distribution on mechanical and sensory properties, taking oral processing into account, are described. To cover a broad range of relevant food textures, we included in our studies two food categories: liquid ("drinkable") and semi-solid ("chewable") fat-containing foods. To allow control over as many properties as possible, our studies were performed in model foods of known composition. As model foods for these food categories, we chose oil-in-water (o/w) emulsions and emulsion-filled gels.

In **Chapter 2**, we introduce charge-based and cross-linking-based methods as feasible methodologies to cluster emulsion droplets. In **Chapter 3**, we investigate the effect of the electrostatic clustering of oil droplets in o/w emulsions on sensory perception. In **Chapter 4**, we assess the role of oil droplet clustering in emulsion-filled gels. Two methods are introduced by which a controlled inhomogeneity at μm- and mm-scale can be achieved in emulsion-filled gels, and their effects on mechanical properties and sensory perception are evaluated. In **Chapter 5**, strongly interacting oil-droplet clusters are studied for their effect on sensory properties in both emulsions and emulsion-filled gels. In **Chapter 6**, clustering of oil droplets as a fat-reduction strategy is assessed in a protein gel. In **Chapter 7**, we study the effects of attractive droplet-droplet interactions and clustering on high volume fraction emulsions and their rheology. In **Chapter 8**, we show how a simple tribological setup can be used to determine the friction behaviour of model gels, but also real foods and model boli. We provide examples of the applicability of this methodology by showcasing the strong effect of, *e.g.* dispersed oil droplet on the surface friction of gels. In the context of this research, this methodology is highly relevant, as we identified a lack of methods to characterise complete solid foods and food boli of solid foods. As we show in this chapter, oil droplet properties not only change the mechanical properties of gels but also change the friction forces between model oral surfaces and foods. In **Chapter 9**, a general discussion and insights from a case-studies investigating the effect of clustering on droplet coalescence are provided. Finally, guidelines for industrial application of the here presented research are included, and recommendations for further research are listed

# Chapter 2 - Clustering of oil droplets in o/w emulsions: Controlling cluster size and interaction strength

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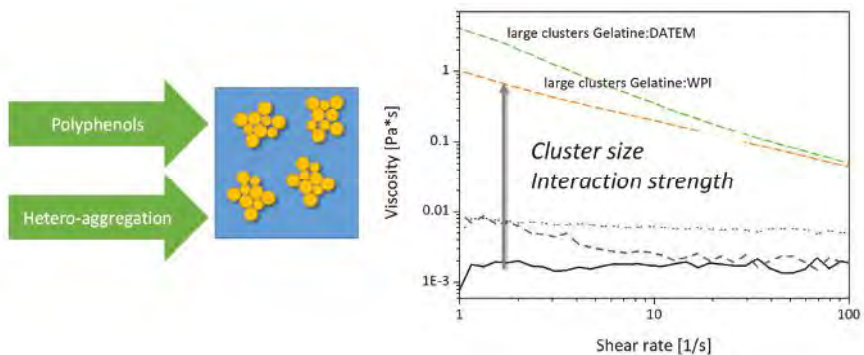
## Abstract

Clustering of oil droplets changes the rheological properties of oil-in-water (o/w) emulsions and can be used as a tool to structure foods. The aim of this study was to manipulate both oil droplet cluster size and cluster strength in liquid o/w emulsions and to investigate the effect of these parameters on the rheological properties. Clustered emulsions were prepared using three different methods: (i) clustering by protein-proanthocyanidin interactions, (ii) clustering by hetero-aggregation of oppositely-charged emulsion droplets, and (iii) enzymatic clustering of protein-stabilised droplets using transglutaminase. Clustering by protein-proanthocyanidin interactions allowed to control oil droplet cluster size from 1 to 140 µm. Clusters decreased in size upon both an increase or decrease in pH but were stable against changes in ionic strength. Hetero-aggregation of oppositely-charged oil droplets (gelatine/whey protein and gelatine/DATEM) allowed to control cluster size from 1 to 40 µm. Clusters showed a strong decrease in size in response to changes in pH and a small decrease in size with increasing ionic strength. Enzymatic clustering did not allow to control cluster size. Cluster strength of proanthocyanidin-stabilised clusters was found to be higher than that of hetero-aggregated clusters. Stabilisation of clusters was likely induced by different protein-proanthocyanidin interactions such as H-bridges, π-π stacking, and hydrophobic interactions, whereas hetero-aggregation is based on electrostatic interactions. Upon clustering, emulsion viscosity increased by up to three orders of magnitude. We conclude that protein-proanthocyanidin interactions and hetero-aggregation are effective methods to tune droplet cluster size and strength in o/w emulsions, and that cluster size and interaction strength control the rheological properties of o/w emulsions with clustered oil droplets.

## 2.1 Introduction

Fat is an essential ingredient of many foods, such as milk, yoghurt, and mayonnaise. Fat composition and oil droplet size largely influence the rheological properties of emulsions and therefore have a large effect on sensory perception of o/w emulsion-based foods. A critical parameter for the rheological properties of o/w emulsions is the spatial distribution of oil droplets (Mao & McClements, 2012b; Mosca, Rocha, Sala, van de Velde, & Stieger, 2012; Sala, van Vliet, Cohen Stuart, Aken, & van de Velde, 2009; Santagiuliana, Piqueras-Fiszman, van der Linden, Stieger, & Scholten, 2018). The spatial distribution of oil droplets in an o/w emulsion can be uniform (homogenous), or non-uniform (inhomogeneous) with macro- or microscopic oil-enriched and oil-depleted areas (Oliver, Berndsen, van Aken, & Scholten, 2015b; Oliver, Wieck, & Scholten, 2016). On a microscopic scale, an inhomogeneous spatial distribution of oil droplets is obtained when the oil droplets cluster. Here, clustering is referred to as the purposefully obtained assembly of multiple oil droplets into clusters with specific and controllable cluster size. Clustering of oil droplets has been shown to cause significant changes in the rheological properties of liquid emulsions (Mao & McClements, 2011). There are several reasons for these changes in rheological properties resulting from droplet clustering. The entrapment of the continuous aqueous phase in the oil droplet cluster causes a strong increase in viscosity in liquid systems since the effective volume fraction of the oil phase increases to a large extent (McClements, 2012). Furthermore, clusters of oil droplets are typically non-spherical objects, which increases the hydrodynamic diameter. As non-spherical objects occupy larger volumes than spherical objects (Marangoni, 2000), the viscosity increases. Additionally, clustering of oil droplets can influence the effective stiffness of oil droplets, as droplet-droplet interactions are introduced. In clustered droplets, additional elasticity is conferred due to bonds between droplets and droplet-droplet friction (Filip et al., 2006). The clustering of oil droplets thus changes the structure and rheological properties of o/w emulsions. Modulation of oil droplet clustering can thus be used as an approach to tailor rheological properties of liquid oil-containing foods, such as emulsion-based beverages (*i.e.* milk drinks), salad dressings and cream liqueurs, and might consequently be used to reformulate foods. It is desired to control cluster size and interaction strength within a cluster and between clusters to obtain o/w emulsions with defined rheological properties.

To induce droplet clustering, several approaches seemed promising and were investigated in this study. Oil droplets are frequently stabilised by proteins, and protein-protein interactions can be used to cluster oil droplets. When proteins are located at the o/w interface of oil droplets, clustering between oil droplets can occur, provided that the electrostatic repulsion between droplets is overcome by attractive interactions. Furthermore, the occurrence of clustering depends on the volume fraction of the oil droplets. With increasing volume fraction of oil droplets, clustering is facilitated due to a higher probability of droplet-droplet contacts and interactions. Specific compounds can induce or facilitate protein-protein interactions. Various compounds belonging to the group of proanthocyanidins have been shown to induce complexation with proteins, such as whey proteins or caseins, which leads to clustering of the proteins (Bohin et al., 2014; Bohin, Vincken, Van Der Hijden, & Gruppen, 2012). There is a broad range of specific interactions responsible for complexation ranging from hydrogen bonds, hydrophobic interactions to π-π stacking and covalent bonds. Currently, it is not known whether proanthocyanidins can induce oil droplet clustering of protein-stabilized droplets. Protein-protein interactions can also be induced by enzymatic cross-linking. Peroxidase has been reported to be able to crosslink proteins, as well as proteins with other polymers (Liu et al., 2015, 2017; Matheis & Whitaker, 1984). Also, transglutaminase can create bonds

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between proteins (Gaspar & de Góes-Favoni, 2015; Zeeb et al., 2013), so it seems plausible that clusters of protein-stabilized oil droplets can be obtained using transglutaminase.

Protein-protein interactions can further be induced by attractive electrostatic interactions. A concept that uses charge-based interactions and allows to cluster oil droplets in o/w emulsions is hetero-aggregation (Mao & McClements, 2011). The mechanism of hetero-aggregation is based on combining oil droplets of the opposite charge. Emulsion droplets with opposite charges are obtained using different types of surface-active agents, such as proteins or charged surfactants. Upon mixing, the opposite charges attract each other leading to the formation of oil droplet clusters. A number of emulsifier combinations for the formation of hetero-aggregates have been reported, such as ß-lactoglobulin and lactoferrin (Iqbal, Hameed, Baloch, & McClements, 2013; Mao & McClements, 2011, 2012a), whey protein isolate and modified starch (Mao & McClements, 2013a, b), and whey protein isolate and saponins (Maier, Zeeb, & Weiss, 2014; Maier, Oechsle, & Weiss, 2015). This approach has been used to prepare and characterize emulsions. However, knowledge on the relationship between specific cluster parameters like cluster strength and morphology and rheological properties of the emulsions is still scarce. This study aimed to develop knowledge on how to manipulate both oil droplet cluster size and cluster strength in liquid o/w emulsions. Controlling these properties is a way to control the properties of emulsions. Emulsions were characterized for cluster strength, size, morphology, and rheological properties. Different new sets of emulsifiers for hetero-aggregation were used to investigate the effect of size and interaction strength within and between clusters. Combinations of whey protein and gelatine, as well as diacetyl tartaric acid ester of mono- and diglycerides (DATEM) and gelatine were used. The influence of pH and ionic strength on cluster size was determined for clustered o/w emulsions

## 2.2 Materials and methods

### 2.2.1 Materials

Whey protein isolate (BiPRO, WPI) was obtained from Davisco (Lot # JE 062-3-420, USA). Powdered sodium caseinate (Na-Cas, EM7) were kindly provided by Friesland Campina (The Netherlands). Gelatine Type 250 PS 30 was acquired from Rousselot (Lot #1207647, The Netherlands). DATEM (combinations of diacetyl tartaric acid ester of mono- and diglycerides) was kindly provided by CP Kelco (USA). Vitaflavan® (grape seed extract) produced by LES DÉRIVÉS RÉSINIQUES ET TERPÉNIQUES (Dax, France) was used as a source of procyanidin. Vitaflavan® contains 78.4% procyanidins, of which less than 25% are monomers, and 35% are di- or trimers. A procyanidin porter content of 65% has been reported by the producer. Anhydrous citric acid (p.a) and sodium hydroxide (p.a.) were acquired from Sigma Aldrich (St. Louis MO, USA). Ethanol (96%) was obtained from VWR (Radnor PA, USA). Sunflower oil (Reddy, The Netherlands) was obtained from a local retailer. Transglutaminase (type WM) was kindly provided by Ajinomoto (Ajinomoto Omnichem, Belgium). Demineralised water was used for all experiments (MiliQ® system, Merck Millipore, Germany).

### 2.2.2 Preparation of clustered oil droplets in o/w emulsions

WPI-stabilised o/w emulsions with proanthocyanidins

An overview of the prepared o/w emulsions can be found in Table 1.

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**Table 1. Overview of the composition of all o/w emulsion clustered by proanthocyanidins-protein interactions and by hetero-aggregation. All o/w emulsions were prepared at oil concentrations of 5, 10 and 20%.**

| Clustering with proanthocyanidins | | | | | |
|---|---|---|---|---|---|
| Emulsions | cluster | final oil concentration [%] | final emulsifier concentration [g/L emulsion] | emulsifier | GSE concentration [% per g protein] |
| *non-clustered emulsion* | | | | | |
| non-clustered emulsion | homogeneous droplet distribution | 5, 10, 20 | 0.9 (5% oil) 1.7 (10% oil) 3 (20% oil) | WPI | 0 |
| *clustered emulsions* | | | | | |
| GSE25 | small cluster | 5, 10, 20 | 0.9 (5% oil) 1.7 (10% oil) 3 (20% oil) | WPI | 25 |
| GSE50 | medium-sized cluster | | | | 50 |
| GSE75 | large cluster | | | | 75 |

| Hetero-aggregated emulsions | | | | | | Volume emulsion (to total 100 mL) [mL] | | |
|---|---|---|---|---|---|---|---|---|
| Emulsions | ratio emulsions | final oil concentration [%] | final emulsifier concentration [g/L emulsion] | emulsifier | | Gelatine | DATEM | WPI |
| *non-clustered emulsions* | | | | | | | | |
| Gelatine:DATEM | 00:10 | 5, 10, 20 | 0.2 (5% oil) 0.4 (10% oil) 0.8 (20% oil) | DATEM | | 0 | 100 | - |
| | 10:00 | | 1.5 (5% oil) 3 (10% oil) 6 (20% oil) | Gelatine | | 100 | 0 | - |
| *clustered emulsions* | | | | | | | | |
| Gelatine:DATEM | 01:09 | 5, 10, 20 | . | Gelatine | DATEM | 10 | 90 | |
| | 02:08 | | | | | 20 | 80 | - |
| | 03:07 | | | | | 30 | 70 | - |
| | 04:06 | | | | | 40 | 60 | - |
| | 05:05 | | | | | 50 | 50 | - |
| | 06:04 | | | | | 60 | 40 | - |
| | 07:03 | | | | | 70 | 30 | - |
| | 09:01 | | | | | 90 | 10 | - |
| *non-clustered emulsions* | | | | | | | | |
| Gelatine:WPI | 00:10 | 5, 10, 20 | 0.5 (5% oil) 1 (10% oil) 1.9 (20% oil) | WPI | | 0 | - | 100 |
| | 10:00 | | 1.5 (5% oil) 3 (10% oil) 6 (20% oil) | Gelatine | | 100 | - | 0 |
| *clustered emulsions* | | | | | | | | |
| Gelatine:WPI | 01:09 | 5, 10, 20 | . | Gelatine | WPI | 10 | - | 90 |
| | 02:08 | | | | | 20 | - | 80 |
| | 03:07 | | | | | 30 | - | 70 |
| | 04:06 | | | | | 40 | - | 60 |
| | 05:05 | | | | | 50 | - | 50 |
| | 06:04 | | | | | 60 | - | 40 |
| | 07:03 | | | | | 70 | - | 30 |
| | 09:01 | | | | | 90 | - | 10 |

O/w emulsions were prepared with an aqueous phase containing WPI (3.75, 1.88 and 0.94 g/L final WPI concentration in the aqueous phase) in a 0.12 M McIlvaine buffer at pH 3. This pH was previously identified to be the optimal pH for interactions between certain proanthocyanidins and proteins (Bohin et al., 2014) (Naczk, Oickle, Pink, & Shahidi, 1996; Rawel, Meidtner, & Kroll, 2005). After complete dissolution, sunflower oil was added to WPI solutions. O/w emulsions had a final oil concentration of 5%, 10% and 20% (v/v). The mixtures were pre-emulsified with a rotor-stator homogeniser (Ultra-Turrax, T25, IKA, Germany) at 8000 rpm for 3 min. Subsequently, pre-emulsions were homogenised at 180 bar for 4 cycles (LabhoScope, Delta Instruments, The Netherlands). As a source of proanthocyanidins, grape seed extract Vitaflavan® was used. Clustered emulsions were prepared by combining various volumes of grape seed extract (GSE) stock solutions (200 g/L GSE) with WPI-stabilised o/w emulsions (5, 10 or 20% (v/v) oil). Between 0.25 g and 0.75 g GSE per gram emulsifying protein (GSE concentration in the final emulsion) was used to obtain emulsions varying in cluster size. We denote this as 25%, 50% and 75% GSE. Dilution effects due to the addition of GSE stock solutions were considered negligible. Following the addition of GSE stock solutions, o/w emulsions were vigorously mixed, by shaking, and stored for 24 h at room temperature to allow oil droplet clustering to occur.

### Hetero-aggregation of WPI stabilised o/w emulsions

An overview of the prepared o/w emulsions can be found in Table 1. First, stock solutions of WPI, gelatine, and DATEM were prepared. WPI at a concentration of 6.4 g/L was added to a 7.5 mM citric acid solution at pH 7 (set with 1 M NaOH). Citric acid was used as a food-grade chelating agent to avoid the influence of divalent ions on clustering. The solution was stirred with a magnetic stirrer for at least 2 h to allow complete hydration of the protein. Gelatine solutions were prepared at a concentration of 20 g gelatine/L aqueous phase by addition of gelatine to a citric acid solution of pH 5 and 7 (set with 1 M NaOH). The gelatine dispersion was heated to 80°C for 30 min while stirring to dissolve the gelatine. DATEM solutions were prepared by dissolving DATEM into sunflower oil at a concentration of 4 g/L oil. Solutions were heated to 80°C for 10 min to dissolve DATEM. Both gelatine and DATEM solutions were cooled to room temperature before further use. The pH of the gelatine solutions was set, if required, to values of 5 or 7 with a 1 M NaOH solution. Aqueous and oil phases were combined to prepare single emulsions with an oil volume fraction of 0.4. The mixtures were pre-emulsified and emulsified as described in 2.2.1. Emulsions were subsequently diluted to final oil concentrations of 5, 10 and 20% (equal to an oil volume fraction of 0.05, 0.10 and 0.20). Hetero-aggregated emulsions were prepared by combining two single emulsions under stirring at room temperature at different volumetric ratios. The selected volume ratios were 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 9:1, and 10:0. Gelatine-stabilised emulsions (positively charged) were mixed with either WPI- or DATEM-stabilised (negatively charged) o/w emulsions. Emulsions were subsequently mixed by slowly rotating the container (Greiner Bio-one, Austria) and stored for 24 h at room temperature before further analysis.

### Transglutaminase-induced clustering of o/w emulsions

WPI-stabilised and caseinate-stabilised o/w emulsions with an oil concentration of 10 and 20% (oil volume fraction of 0.10 and 0.20) were prepared. The emulsifiers were dissolved in water at a concentration of 10 g/L. The pH was adjusted to pH 7 with 1 M NaOH or HCl if required. After dissolving, the emulsions were prepared as described in 2.2.1. The final concentrations of WPI were, therefore, 1.5 g/L (10%) and 3 g/L (20%). Transglutaminase was pre-dissolved as a stock solution at a concentration of 0.32 g/mL in Milli-Q water. Transglutaminase was added in various ratios of the enzyme to emulsifying protein (1:20, 1:10 and 1:5). After vigorous mixing, transglutaminase-treated emulsions were incubated for 1 to 24 hours at 37 °C. The reaction was stopped by heating the mixture to 86 °C for 10 minutes and cooling to room temperature on ice. The emulsion was stored for 24 h at room temperature before further analysis.

### 2.2.3 Physical characterisation of o/w emulsions

### Determination of particle size and effective cluster size

The size of oil droplets in single emulsions was determined by static light scattering (Master sizer 2000S, Malvern Instruments Ltd., UK) and microscopy. The refractive index of the oil droplets was set as 1.45 and that of the dispersant at 1.33. Pump speed was set at 500 rpm. Droplet size was reported as d0.5, D[4,3] and D[3,2]. Each measurement was performed in triplicate. For size measurements by microscopy, emulsions were diluted with their corresponding aqueous phase (without emulsifier), placed on a microscope slide, and covered with a coverslip. Each sample was analysed at six positions using an optical light microscope (Axioskop 2 plus, Carl Zeiss AG, Germany) equipped with a camera (Axiocam ERc 5S, Carl Zeiss AG, Germany) and Visio imaging software. Recorded images were analysed for particle size (area)

using ImageJ. To scale from pixel to μm, the scale of a microscopic picture (based on the built-in ZEN imaging software of the Axioskop 2 plus) was used. The brightness was adjusted automatically, and a threshold of <0.2 μm was applied to remove background noise. An effective cluster size was calculated from the droplet area, assuming a spherical shape. At least three independent emulsion replicates were used, probed at six positions, and an average diameter with standard deviation calculated and reported. Clustered emulsions cross-linked by proanthocyanidins and transglutaminase were also analysed for cluster size using static light scattering (Malvern Mastersizer 2000S, Malvern Instruments Ltd., UK). For the o/w emulsions clustered using charge-based clustering, clusters disintegrated during the static light scattering measurements due to insufficiently strong interactions. Therefore, cluster size could not be determined by light scattering and was assessed by image analysis as described before. The cluster size was reported as effective cluster size with standard deviation.

Determination of ζ -potential
The zeta potential was determined using a Zetasizer Nano ZS series (Malvern Instruments, Worcestershire, UK). Single and clustered emulsions were diluted at least 100x with the corresponding aqueous phase (without emulsifier). Each emulsion was measured in triplicate at 20°C.

Stability of clustered o/w emulsions at different pH and ionic strength
Clustered emulsions were tested for stability under different environmental conditions, *e.g.* different pH and ionic strength. The pH of the emulsions was adjusted from 3 to 8 using HCl-solutions (1 M) or NaOH-solutions (1 M). Ionic strength was altered by adding NaCl solutions (5 M) achieving an ionic strength range from 0 mM to 180 mM. Following a storage time of 24 h at room temperature, o/w emulsions were reanalysed for cluster size, so stability is expressed as changes in cluster size.

Rheological characterisation
Rheological tests were conducted with an Anton Paar 302 Rheometer (MCR 302, Anton Paar GmbH, Graz, Austria). For determination of flow curves, a concentric cylinder cup geometry (sandblasted) (CC17-S) was used (probe diameter: 16.654 mm, cup inner diameter: 18.077 mm). Viscosity measurements were carried out with an increasing logarithmic shear rate ramp from 1-1000 s-1 within 10 minutes at 22°C, the temperature being controlled with a Peltier element and a water bath. Viscosities at high shear rates should be considered with care, as time effects to reach equilibrium were not taken into consideration.

To determine cluster strength, the critical strain and stress at critical strain were determined. The elastic modulus of the emulsions, G', was measured by oscillatory tests with a parallel plate geometry (PP50) (diameter 5 cm). The gap height was set to 1 mm. Amplitude sweeps were conducted at a frequency of 10 rad/s at a strain range of 0.01-100%. The critical strain was taken from the amplitude sweeps where the elastic modulus G' deviated by more than 5% from the G' in the linear viscoelastic region (LVR). This deviation is considered representative of a structural breakdown of the clustered emulsions. We assume that with increasing cluster strength, thus higher interaction strength, the structural breakdown occurs at higher applied deformations. Frequency sweeps were performed from 0.016 to 15.92 Hz at a strain of 0.5% (or 0.1% where necessary), which was within the linear viscoelastic region (LVR) of each emulsion.

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### 2.2.4 Estimation of effective oil volume fraction of clustered o/w emulsions

The effective volume fraction of clustered o/w emulsions was roughly estimated using the Krieger-Dougherty equation (Krieger & Dougherty, 1959; Luckham & Ukeje, 1999),

$$\frac{\eta_{eff}}{\eta_0} = n_r = \left(1 - \frac{\phi}{\phi_m}\right)^{-2.5*\phi_m} \qquad 1$$

in which $n_r$ is the relative viscosity, defined as the ratio of effective viscosity, $\eta_{eff}$, of the clustered emulsion and the viscosity of the individual droplet emulsions, $\eta_{0,}$. The intrinsic viscosity $[\eta]$ was assumed to be 2.5 (McClements, 2015) and the maximum packing fraction, $\phi_m$, as 0.64 (Genovese, Lozano, & Rao, 2007; Oliver et al., 2015b). The viscosity values of the clustered emulsions were taken at a shear rate of 50 s-1, as the determination of the zero-shear viscosity was not very reliable due to larger standard deviations at low shear rates. The viscosities at a shear rate of 50 s-1 were used to estimate the volume fraction of the clustered emulsions, $\phi$, which can be seen as an effective volume fraction. It has to be noted that the estimation of the volume fraction of the clustered emulsion violates several assumptions of the Krieger-Dougherty equation, such as the hard-sphere interaction potential, spherical particle shape and monodispersity of particle size. Thus, we emphasize that the volume fraction of the clustered emulsions discussed in the following sections is only a rough estimate.

To estimate the effective volume fraction of clustered emulsions using an alternative methodology, a calibration curve was measured, which linked viscosity of individual droplet o/w emulsions to total oil volume fraction ("calibration curve"). To obtain this calibration curve, a set of 5 emulsions with an oil concentration ranging from 5 to 60% (volume fraction of 0.05 to 0.60) were prepared. The experimental data were fitted to an exponential function

$$\phi = a * \eta_{(50s^{-1})}{}^b \qquad 2$$

where $\phi$ represents the oil volume fraction, $\eta_{(50s^{-1})}$ the apparent viscosity at a shear rate of 50s-1, and a and b are fitting parameters. This volume fraction was taken as an estimate of the effective volume fraction of clustered o/w emulsions and compared to the above-described estimation of the effective volume fraction of clustered o/w emulsions based on the Krieger-Dougherty equation.

## 2.3 Results and discussion

### 2.3.1 Oil droplet clusters of WPI-stabilised o/w emulsions with proanthocyanidins

By using the grape seed extract (GSE), Vitaflavan® as a proanthocyanidin source at pH 3, concentration-dependent clustering of oil droplets of WPI-stabilised o/w emulsions was observed (Figure 1).
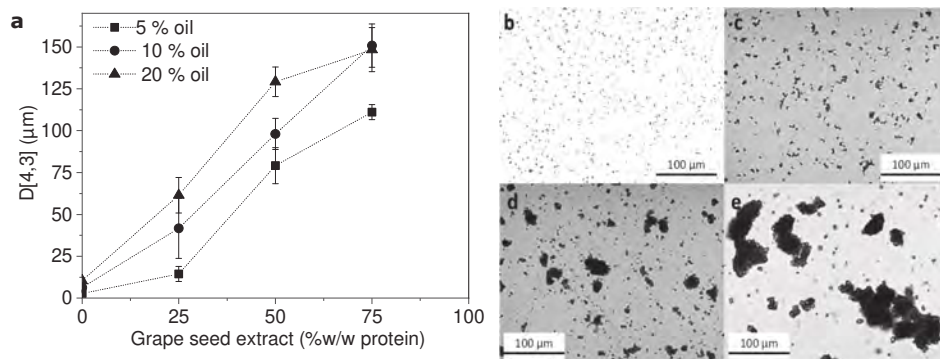
**Figure 1. a) Oil droplet cluster size (D(4,3)) as a function of grape seed extract concentration for WPI stabilised o/w emulsions varying in oil content. Lines are added to guide the eye (n=3, error bars indicate standard deviation). Right images: Micrographs of emulsions. The images show a non-clustered o/w emulsion (b), an emulsion clustered using 25% grape seed extract (c), an emulsion clustered using 50% grape seed extract (d) and an emulsion clustered using 75% grape seed extract (e).**

Cluster size strongly increased with increasing GSE concentration. Starting with a single-droplet emulsion with oil droplets of around 1 μm diameter, the obtained cluster sizes ranged from 14 μm for 25% grape seed extract per gram whey protein to 140 μm for 75% grape seed extract per gram whey protein. 25% grape seed extract per gram whey protein was found to be the minimum concentration required to cluster oil droplets.

The oil volume fraction of the emulsion affected cluster size in a non-systematic manner. For an increase in oil concentration from 5% to 20%, an increase in cluster size was observed. A decrease in distance between droplets by increasing volume fraction led to the formation of larger clusters. The formation of clusters and the efficiency in droplet clustering are a result of a variety of interactions occurring. It is known that proanthocyanidins present in grape seed extract vary in composition from mono- to heptameric oligomers (Bohin et al., 2012). Due to the large diversity in oligomers, a variety of physical and chemical interactions can occur, leading to the formation of hydrogen bridges as well as covalent bonds. Bohin et al. (2012, 2014) argued that the complexation of β-lactoglobulin with proanthocyanidins is based on an extended hydrogen bond network. We suggest that such interactions are also responsible for the clustering of WPI-stabilised o/w emulsion droplets by proanthocyanidins present in grape seed extract since WPI contains β-lactoglobulin as the main constituent.

Additionally, the presence of proanthocyanidins has been suggested to alter the unfolding of globular proteins leading to exposure of hydrophobic side groups (Kanakis et al., 2011). Hydrophobic interactions and entanglements of unfolded protein strands increase the interactions and network formation between proteins, thus further stabilising clusters. Next to hydrogen bonds and hydrophobic interactions, also stacking of polyphenolic rings through π-π interactions has been suggested as a possible contribution to the complexation (Charlton et al., 2002; Kanakis et al., 2011). These oil droplet clusters are therefore most likely stabilised by a variety of interactions.

Figure 2 shows the influence of incubation time on cluster size of WPI-stabilised o/w emulsions with 20% oil, clustered with proanthocyanidins.
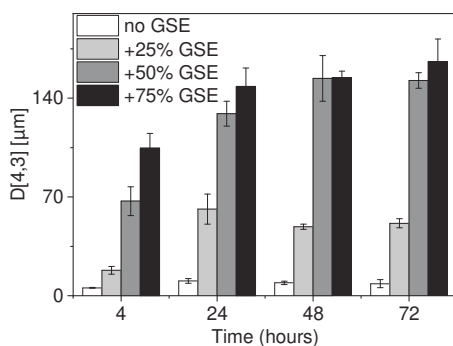


**Figure 2. Oil droplet cluster size (D4,3) as a function of incubation time at room temperature for WPI-stabilised o/w emulsions at 20% oil concentration (n=3, error bars indicate standard deviation).**
Incubation time was varied from 4 to 72 h. The most substantial increase in cluster size was observed within the first 24 h. For a relatively low GSE content (25%, light grey bars), the cluster size increased to 20 μm after 4 h and to 60 μm after 24 h. For higher GSE concentrations (medium and dark grey bars), an increase in time from 4 to 24 h led to an increase in cluster size from 65 to 130 μm for 50% GSE per g protein, and from 100 to 165 μm for 75% GSE. However, at an additional increase in incubation time from 24 to 72 h, cluster size levelled off for higher concentrations of GSE and for low concentrations even seemed to decrease slightly. Consequently, the incubation time for further experiments was kept at 24 h.

We evaluated the stability of oil droplet clusters, expressed as cluster size, against changes in pH and ionic strength within 24 hours. We would like to add that we evaluated the stability of the clusters, rather than the stability of the emulsions itself. Due to the presence of clusters, creaming of the emulsions occurred, but this was reversible upon gentle shaking. The clusters themselves did not change in this process. We related the final cluster size to the initial size immediately after preparation of the clusters (cluster size$_{end}$ / cluster size$_{initial}$). An overview of the changes in cluster size upon changes in pH and ionic strength can be found in Figure 3.
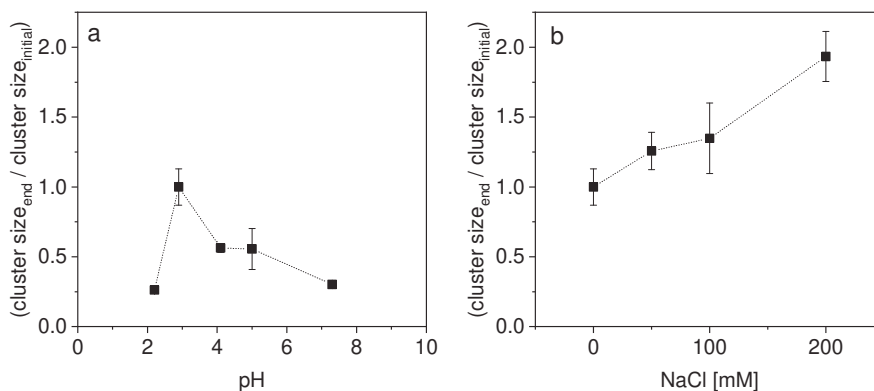
**Figure 3. Relative change in effective diameter (cluster size$_{end}$/cluster size$_{initial}$) of clustered emulsions as a function of changes in pH (a) and NaCl concentration (b). Dotted lines are added to guide the eye (n=3, error bars indicate standard deviation).**

Upon both a decrease from pH 3 to 2 and an increase from pH 3 to 8, cluster size decreased. Clusters disintegrated partly into single droplets, indicating a decrease of attractive interactions between the droplets. Cluster size strongly decreased between pH 3 and 5, which is close to the isoelectric point of the proteins. One could expect that the reduced positive charges of the proteins, due to a pH close to the isoelectric point, facilitate clustering due to less electrostatic repulsion. The disintegration is, therefore, not only related to changes in the electrostatic interactions between proteins. A potential reason for the disintegration could be a reduced affinity between whey proteins and proanthocyanidins at higher pH. In the case proteins were still positively charged when the pH was further reduced from 3 to 2, the size also decreased. We attribute this to possible degradation of interflavan bonds, which are covalent bonds between subunits of the proanthocyanidin, or other pH-sensitive bonds (Vidal, Cartalade, Souquet, Fulcrand, & Cheynier, 2002). Hydrogen bonds and π-π interactions may also change with changing pH (Ozdal, Capanoglu, & Altay, 2013). These results suggest that the clusters formed by clustering of WPI-stabilised o/w emulsion droplets with proanthocyanidins are mainly stabilised by non-covalent interactions, as evidence for covalent interactions could not be found.

Cluster size also varied upon changes in ionic strength. Cluster size increased linearly with increasing NaCl concentration from a diameter [D4,3] of 60 μm at standard buffer conditions (no added NaCl) to 100 μm at 200 mM of added NaCl. This was most likely related to a reduction of electrostatic repulsion between the WPI-stabilised o/w emulsion droplet clusters at higher ionic strengths, which allowed further growth of the clusters. These results show that electrostatic interactions are of importance both within the cluster and between clusters. The clusters showed sensitivity to changes in pH, by cluster breakdown, and sensitivity to changes in the ionic strength; cluster size increased with increasing ionic strength.

The effect of droplet clustering on the rheological properties of the emulsions was examined. In Figure 4, examples of flow curves of non-clustered and clustered (75% GSE) o/w emulsions varying in oil concentration (5, 10 and 20%) are presented.
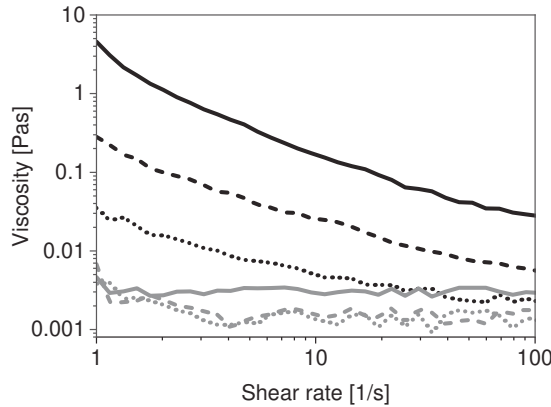
**Figure 4. Exemplary flow curves of non-clustered (grey) and clustered (75% GSE, black) o/w emulsions are varying in oil concentration (5% oil: dotted, 10% oil: dashed and 20% oil: full line).**

Non-clustered oil droplet emulsions showed Newtonian flow behaviour, with a viscosity of around 0.001 to 0.01 Pa·s. Upon clustering of the WPI-stabilised emulsions (5, 10 and 20% oil concentration) viscosity increased and displayed strong shear thinning behaviour. For the largest clusters stabilised with 75% GSE (w/w protein) and with an oil concentration of 20%, the viscosity increased by at least a factor 100 at low shear rates to values > 1 Pa·s. The increase in viscosity was an effect of the increase of the effective oil volume fraction because of the oil droplet clustering (Montesi, Peña, & Pasquali, 2004; Starov & Zhdanov, 2003). The larger the oil droplet clusters at a certain absolute oil volume fraction, the higher the effective volume fraction of the droplets due to entrapment of the bulk liquid between oil droplets within the clusters. Wu and co-workers showed comparable findings, in terms of rheological behaviour, in clustered emulsions using divalent salts, indicating an increasing viscosity with increasing cluster size (Wu, Degner, & McClements, 2013). Furthermore, the presented results are in line with studies on non-controlled, depletion induced clustering of droplets, where a considerable increase in viscosity and shear thinning behaviour were reported (Berli, Quemada, & Parker, 2002; Dickinson & Golding, 1997).

In Figure 5, the effective volume fraction of clustered o/w emulsions, estimated roughly using the Krieger-Dougherty model (Equation 1), is shown as a function of the effective size of droplet clusters (filled symbols).
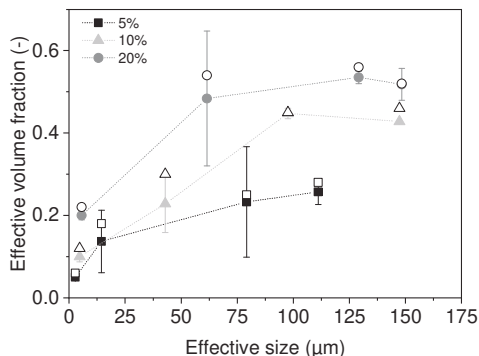
**Figure 5. The effective volume fraction of WPI-stabilised o/w emulsion clustered with grape seed extract (25%, 50% and 75% GSE per g protein) as a function of cluster size for oil volume fractions of 0.05, 0.10 and 0.20 (n=3, error bars are standard deviation). Closed symbols: estimates of effective volume fraction obtained with Krieger-Dougherty model; open symbols: estimates of effective volume fraction obtained from an "individual droplet calibration curve".**

With increasing cluster size, the amount of entrapped continuous phase was enhanced, and the effective volume fraction increased. For a 0.05 oil volume fraction (5%, squares), the effective volume fraction increased to 0.25. In the case of an oil volume fraction of 0.20 and for the largest cluster sizes, the effective oil volume fraction of the clustered WPI-stabilised emulsions increased to values of around 0.55. We emphasize again that the estimated effective volume fraction of the clustered emulsions obtained using the Krieger-Dougherty equation may only be a crude estimate as assumptions regarding droplet shape and droplet size distribution are violated. A better estimate of the intrinsic viscosity and including the droplet polydispersity could be improvements in this approach. However, these aspects are out of the scope of this study.

The values of effective volume fraction obtained using the Krieger-Dougherty model were compared to estimates of effective volume fraction obtained by the "calibration curve", as explained in paragraph 2.4. Figure 5 shows that both estimations gave comparable effective volume fractions, indicating that the Krieger-Dougherty model can be used despite its indicated limitations.

Besides the entrapment of bulk phase within the clusters, also other factors should be considered when discussing the increase in viscosity upon clustering. The clustering process of oil droplets creates clusters with a non-spherical shape. The presence of such anisotropic clusters hinders flow, reduces cluster mobility, and thus further increases the effective volume fraction of the clusters (Mueller, Llewellin, & Mader, 2011). Additionally, the interactions within clusters may also contribute to changes in flow behaviour. The interactions between the droplets determine the mobility of the droplets within the clusters and the cluster stiffness and strength. For weak interactions, the clusters may be able to deform, whereas, for strong interactions, the deformability of the clusters will be reduced (Montesi et al., 2004).

The critical strain and the stress at critical strain were used to estimate the apparent interaction strength or cluster strength (Mancini, Montanari, Peressini, & Fantozzi, 2002). Examples for amplitude sweeps can

be found in the supplementary materials. For emulsions with an oil volume fraction of 0.20, critical strain values of 1.37±0.40% for 25% GSE, 4.30±2.05% for 50% GSE and 6.43±1.30% for 75% GSE were found. The stress at critical strain increased with increasing GSE concentration from 0.2 Pa for 25% GSE to 2.0 Pa for 50% GSE and 8.8 Pa for 75% GSE. We suggest that not only the effective size of the cluster increases with increasing concentration of grape seed extract but also the strength of the clusters. Above a certain total oil volume fraction and GSE concentration, a space spanning network is formed.

To conclude, the use of grape seed extract (proanthocyanidins) allows clustering of WPI-stabilised o/w emulsion droplets with varying oil droplet cluster size. The size of oil droplet clusters and the stability of the clusters depend on the concentration of grape seed extract and consequently on proanthocyanidin concentration. The interactions are most likely electrostatic interactions or H-bonds. These interactions affect the clustering strength, which, together with increased cluster size, affect the rheological behaviour of the emulsions.

### 2.3.2 Hetero-aggregation of o/w emulsions

To obtain clusters stabilised by charge-based interactions, emulsion droplets with an initial size of around 2 µm were clustered with hetero-aggregation. Two sets of emulsions with oil droplets stabilised with oppositely-charged emulsifiers were used. The emulsifier combinations were (1) gelatine (pI = 8-9) and WPI (pI = 4.5) at pH 7 and (2) the novel combination for hetero-aggregation gelatine and DATEM (pKa = 3) at pH 5. DATEM-stabilised emulsions were negatively charged, with a constant zeta potential of around -60 mV over a pH range from 3 to 7. WPI-stabilised emulsions displayed an average zeta potential of 0 mV at pH 5, close to the pI of WPI, a positive charge (+25 mV at pH 3) below the pI and a negative charge (-48 mV at pH 7) above the pI. Gelatine (pI around 8-9) showed a slightly positive charge of +10mV at pH 5 and of +7mV at pH 7. To obtain hetero-aggregated emulsions with a large zeta-potential difference (Δζ), gelatine emulsions were used as a source for the positively- charged oil droplets.

These droplets were mixed with either negatively-charged WPI-stabilised droplets at pH 7, or DATEM-stabilised droplets at pH 5. When combining the different single emulsions in various mixing ratios, a broad range of cluster sizes was obtained (Figure 6).
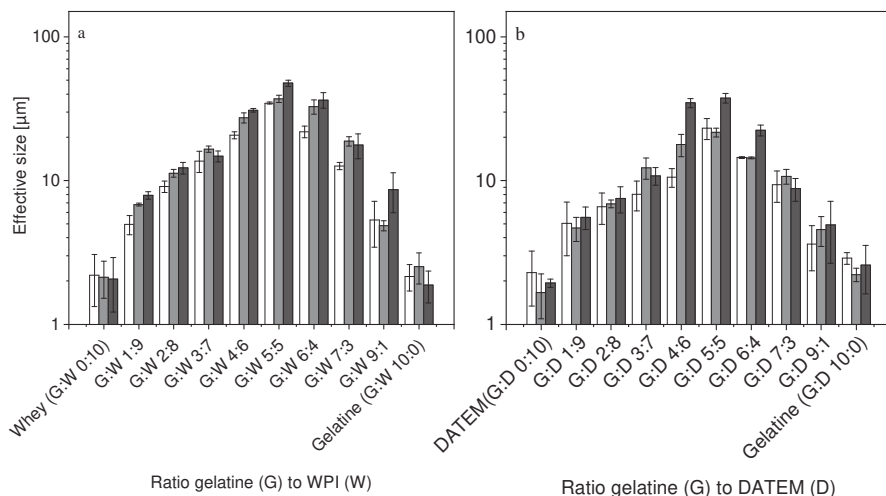
**Figure 6. Effective size of hetero-aggregated o/w emulsions as a function of mixing ratio of oppositely charged o/w emulsions. a) Hetero-aggregates prepared by mixing gelatine and WPI-stabilised emulsions at pH 7. b) Hetero-aggregates prepared by mixing gelatine- and DATEM-stabilised emulsions at pH 5. Effective diameter is shown as function of oil concentration at 5% (white), 10% (grey) and 20% (black). n=3, error bars indicate standard deviation**
The mixing ratio strongly affected the effective cluster size of clustered emulsions. A large increase in cluster size with increasing oil volume fraction was observed for gelatine-WPI hetero-aggregated emulsions (Figure 6a). In contrast, a smaller increase was observed for the gelatine-DATEM hetero-aggregated emulsions (Figure 6b). Both emulsions with hetero-aggregated clusters exhibited a maximum cluster size at a mixing ratio of 5:5. For the gelatine-WPI hetero-aggregated emulsion with an oil volume fraction of 0.2 at a ratio 5:5, cluster size was around 50 µm (Figure 6a), which represented a large increase from the single droplet diameter of 2 µm. The corresponding gelatine-DATEM emulsion displayed a cluster size of around 40 µm (Figure 6b). Cluster size tended to increase to a small extent with increasing oil volume fraction. The dependency of cluster size on mixing ratio can be explained based on the charge stoichiometry; an equal quantity of positively- and negatively-charged droplets of the same size is necessary to create the largest possible clusters. In the presence of an excess of one of the charged droplets, the cluster growth is limited by the less abundant droplet type. These findings agree with previous studies using different oppositely charged emulsifiers, in which the largest cluster size was found towards intermediate mixing ratio of hetero-aggregated o/w emulsions (Maier, Ensenberger, Irmscher, & Weiss, 2016; Maier et al., 2015; Mao & McClements, 2011, 2012a; Simo, Mao, Tokle, Decker, & McClements, 2012).

As mentioned, gelatine-WPI clusters had a larger size than gelatine-DATEM clusters. This difference in size between the two types of oil droplet clusters can be attributed to differences in the electrostatic attraction between negatively- and positively-charged oil droplets. Based on the differences in electrostatic interactions, it could be expected that the morphology of the clusters differs. We expect that clusters form more compact structures when attractive forces are relatively weak, as rearrangements would be easier.

In contrast, clusters might form more open and irregularly shaped structures when the attractive forces are strong, due to a hindered droplet rearrangement after clustering.

However, as shown in Figure 7, gelatine-WPI clusters (Figure 7a) were more open. They appeared larger than clusters prepared with gelatine and DATEM (Figure 7b), which were smaller, and more compact and spherical.
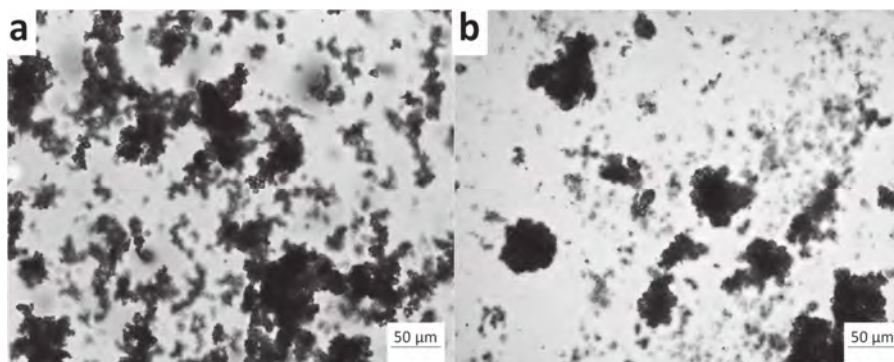


**Figure 7. Micrographs (light microscopy) of two hetero-aggregated o/w emulsions; a) gelatine-WPI hetero-aggregates (5:5 mixing ratio) and b) gelatine-DATEM hetero-aggregates (5:5 mixing ratio).**
Even though the interactions among droplets in gelatine-DATEM clusters are stronger, they are not strong enough to prevent rearrangements. These stronger interactions with possible rearrangements seem to lead to more compact aggregates. Mainly, we suggest that the open and more "cloudy" structures of the gelatine-WPI clusters are an effect of their very weak interactions.

To test the stability of hetero-aggregated clusters against environmental conditions, the ionic strength was varied from 3 mM to 180 mM NaCl and the pH from 3 to 8. Stability was assessed by investigating changes in cluster size by relating the final cluster size to the initial size immediately after preparation of the clusters (cluster size$_{end}$ / cluster size$_{initial}$). A change of pH in the hetero-aggregated emulsion had a large effect on the stability of clusters (Figure 8).
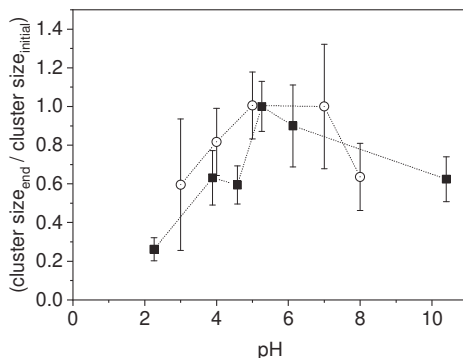
**Figure 8. Relative change in effective diameter (cluster size$_{end}$/cluster size$_{initial}$) of clustered emulsions as a function of changes in pH. Gelatine-DATEM hetero-aggregated o/w emulsions (5:5 mixing ratio) are depicted in black squares and gelatine-WPI hetero-aggregated o/w emulsions (5:5 mixing ratio) in open circles. Dotted lines are added to guide the eye (n=3, error bars indicate standard deviation).**

For both cluster types, the cluster size was largest at the pH of preparation. Gelatine-WPI hetero-aggregated emulsions were prepared at pH 7, and at this pH, the clusters showed the highest stability. When pH was decreased slightly to pH 5, no differences in cluster size were seen yet, and clusters appeared to be stable. However, when the pH was further decreased to pH 3 or increased to pH 8, clusters became more unstable and the effective diameter decreased to 60% of the initial size. Similarly, for the DATEM-gelatine clusters prepared at pH 5, clusters were observed to be most stable at this pH. Cluster size decreased to 20% of their initial cluster size when pH was either increased to 11 or decreased to pH 2. At both low and high pH, gelatine-WPI and gelatine-DATEM hetero-aggregated emulsions decreased in cluster size due to a decrease in electrostatic attraction. In case of gelatine-WPI hetero-aggregated emulsions, at low pH both gelatine and WPI carry a positive charge, leading to electrostatic repulsion, which initiates the disintegration of the clusters. At high pH, gelatine has either a net zero charge or slightly negative charge, and electrostatic interactions are limited. Both DATEM and WPI are negatively charged at high pH. Thus, clusters can disintegrate through electrostatic repulsion. This behaviour indicates the high importance of electrostatic forces in the stabilisation of the hetero-aggregated clusters.

Although pH seems to have a large effect on the stability of clusters, the effect of ionic strength was limited. For both hetero-aggregated emulsions (gelatine-WPI and gelatine-DATEM), the increase in ionic strength up to 180 mM did not change cluster size (data not shown). So even though salt can reduce the electrostatic attraction by charge screening, no disintegration of clusters was observed. The charge difference between the two types of clusters was relatively large, which appeared to result in high stability of clusters towards changes in ionic strength. However, as some clustering is still visible in situations of low electrostatic attraction or electrostatic repulsion, we believe that clusters are closely packed systems, and other attractive interactions, such as van der Waals interactions and hydrogen bonding, may also play a role in their stabilisation, in addition to electrostatic interactions. The clustering of the droplets had a large influence on the rheological properties of the emulsions. Representative flow curves can be found in

Figure 9 showing o/w emulsions with non-clustered and highly clustered emulsions at the tested oil concentrations.
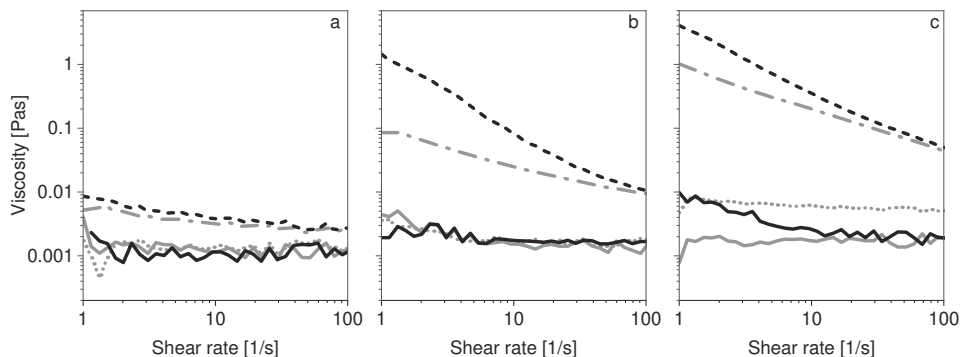


**Figure 9. Flow curves of non-clustered and hetero-aggregated o/w emulsions varying in oil concentration (a: 5%, b: 10%, c: 20%). Black line shows DATEM-stabilised emulsions, the dotted line shows gelatine-stabilised emulsion, the grey line indicates whey-stabilised emulsions, dashed-dotted line depicts large clusters of gelatine-whey emulsions (GW55) and dashed line shows large clusters of gelatine-DATEM (GD55) emulsions.**

Gelatine-stabilised, DATEM-stabilised and WPI-stabilised single-droplet emulsions showed Newtonian flow-behaviour with viscosities ranging from 0.001 to 0.01 Pa·s at volume fractions of 0.05-0.20. Upon clustering the emulsions by hetero-aggregation, the viscosity increased up to about three orders of magnitude, depending on cluster size and cluster type and shear-thinning flow behaviour was introduced. Viscosity increased to about 3 Pa·s for highly clustered emulsions of gelatine-DATEM and 1 Pa·s for gelatine-WPI emulsions at low shear rates (1-10/s).

Reasons for the increase in viscosity have already been discussed previously for grape seed extract clustered emulsions. In this case, we saw that viscosity is not only determined by cluster size but that the cluster properties also play an important role. Even though the gelatine-DATEM clusters were smaller, the viscosity increase was the largest, which indicates that the cluster strength also plays an important role. When comparing our data with the research of Mao and co-workers (2012b, 2013a), the results are in good agreement. The viscosity values measured for hetero-aggregated emulsion in our study were higher than those in the hetero-aggregated systems consisting of modified starch-whey-stabilised clusters (Mao & McClements, 2013a) but comparable to the viscosity values of clusters stabilised by lactoferrin and beta-lactoglobulin (Mao & McClements, 2012a).

We conclude that cluster size and interaction strength, derived from emulsifier charge and –type, determine rheological properties of clustered emulsions at a given oil volume fraction. Figure 10 presents the effective volume fraction estimated using the Krieger-Dougherty model as a function of cluster size for both hetero-aggregated emulsions (gelatine-DATEM and gelatine-WPI) at 0.20 oil volume fraction. With an increasing cluster size, the effective volume fraction increased for both emulsion types. The effective volume fraction increased to about 0.50 for both emulsions, which is 2.5 times larger than the absolute oil volume fraction of 0.20. Similarly, for emulsions with 0.10 oil volume fraction, the effective volume fraction increased to up to 0.50, and thus increased by a factor of 5.
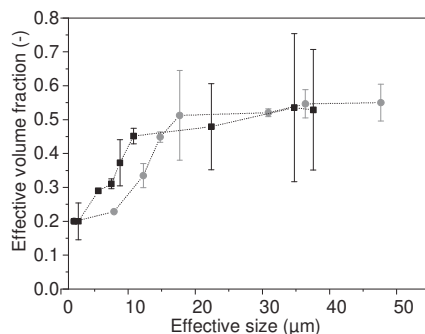
**Figure 10. Effective oil volume fraction as a function of oil droplet cluster size of hetero-aggregated o/w emulsions. Black squares represent gelatine-DATEM hetero-aggregated o/w emulsion with 0.20 oil volume fraction and grey circles represent gelatine-WPI hetero-aggregated emulsions with 0.20 oil volume fraction. Lines are added to guide the eye (n=3, error bars indicate standard deviation)**

As previously discussed, the rheological properties of the clustered emulsions depend on the properties of the clusters. The cluster interaction strength is determined by the strength of the electrostatic interactions. Upon shearing, clusters may be torn apart when the shear force applied is larger than the force holding the cluster together, thereby decreasing the viscosity. Furthermore, cluster-cluster interactions have to be taken into consideration. With increasing total oil-volume fraction, the degree of free movement of the clusters are reduced, which is expected to increase the viscosity. We measured the elastic modulus, G', and estimated the critical strain of the hetero-aggregated emulsions to gain insight into the interaction strength within and between clusters. Emulsions with small clusters, *e.g.* in a mixing ratio 1:9 and 2:8, showed mainly viscous behaviour and the elastic modulus upon increasing strain was insufficiently stable to determine a critical strain. Larger clusters at the same oil volume fraction, *e.g.* with ratios of 4:6, 5:5 and 6:4, showed comparable critical strains. As an example of the difference in cluster type, we discuss the results for clusters of a mixing ratio 4:6. Gelatine-WPI hetero-aggregated emulsions had a critical strain of 1.77±0.60% (and stress at a critical strain of 2 Pa).

In comparison, gelatine-DATEM emulsions with the same ratio exhibited a much larger critical strain of 4.12±0.80 and stress at a critical strain of 6 Pa (examples of measurements can be found in the supplementary material). Based on these values, more and stronger interactions are assumed to be present in gelatine-DATEM hetero-aggregated emulsions than in gelatine-WPI hetero-aggregated emulsions. This is again consistent with the larger difference in charge density between oil droplets, as determined by zeta-potential measurements.

Concluding, hetero-aggregation allows not only a controlled clustering of oil droplets in o/w emulsions in terms of cluster size, but also enables to vary interaction strength within the clusters by emulsifier choice. Both cluster size and cluster strength affect the viscosity of the hetero-aggregated emulsions.

### 2.3.3 Clustering of oil droplets using transglutaminase

To create clusters with strong covalent interactions between the emulsifying proteins, we screened the applicability of transglutaminase as a cross-linking agent for protein-stabilised emulsions. When WPI was

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used as an emulsifier, we observed only very limited or no clustering of oil droplets (with a ratio enzyme: protein of 1:20 and 1:10, at all oil concentrations). This may be due to limited affinity for transglutaminase towards WPI, which has been ascribed in literature to steric inaccessibility of the enzyme to the protein (Ercili-Cura et al., 2012). Flexible proteins, such as caseins, are expected to have a higher affinity towards each other, which might lead to a better clustering of oil droplets stabilised with this protein. Therefore, we also prepared emulsions with Na-caseinate as an emulsifier. For emulsions containing transglutaminase (enzyme: protein ratio of 1:5) and 20% oil, we observed the formation of oil droplet clusters. However, the cluster size increased to values of around 8 μm only after 24 hours of incubation. The efficiency of cross-linking was increased slightly by increasing the ionic strength to 150 mM sodium chloride, resulting in a cluster size of 10 μm (enzyme: protein ratio of 1:5, 24 hours incubation, 20% oil). Compared to the clustering efficiency of the other two methods, *e.g.* hetero-aggregation and GSE-induced clustering, transglutaminase-induced clustering provided a very low clustering efficiency under the tested conditions. By varying the type of protein (whey, casein), enzyme concentration (up to 1:5 emulsifier to enzyme), oil volume fraction (up to 20%) and ionic strength (250mM NaCl), we did not obtain clusters of a similar size range as obtained with hetero-aggregation or clustering with GSE. To improve the efficiency of this method, further research would be required to increase the affinity between the enzyme and the emulsifier to obtain clusters with a controlled size range. Considering that clusters can be tuned efficiently using hetero-aggregation and chemical clustering, we choose to focus on these two methods and discuss the relation between cluster size, cluster strength, effective volume fraction and the ability to increase viscosity.

### 2.3.4 Comparison of hetero-aggregation and clustering with grape seed extract

In this study, we aimed at obtaining oil droplet clusters varying both in cluster size and cluster strength to adjust the rheological properties of emulsions. An overview of the properties of selected samples can be found in Table 2.

**Table 2. Overview of physical/mechanical characteristics – cluster size, viscosity, effective oil volume fraction, of o/w emulsion clustered by proanthocyanidins-protein interactions and by hetero-aggregation. All o/w emulsions shown were prepared at final oil concentrations of 20%.**

| Clustering with proanthocyanidins | Cluster | cluster size [μm] | viscosity (at 50/s) [mPa·s] | effective oil volume fraction [-] |
|---|---|---|---|---|
| *non-clustered emulsions* | | | | |
| *non-clustered emulsions* | homogeneous droplet distribution | 5.6 ± 1.7 | 2.88 ± 0.09 | 0.20 |
| *clustered emulsions* | | | | |
| *GSE25* | small cluster | 61.5 ± 10.6 | 27.50 ± 5.37 | 0.48 |
| *GSE50* | medium sized cluster | 129.2 ± 8.8 | 52.00 ± 0.85 | 0.54 |
| *GSE75* | large cluster | 148.3 ± 13.1 | 40.95 ± 1.77 | 0.52 |
| **Hetero-aggregated emulsions** | Mixing ratio | cluster size [μm] | viscosity (at 50/s) [mPa·s] | effective oil volume fraction [-] |
| *non-clustered emulsions* | | | | |
| *Gelatine:DATEM* | 0:10 | 1.9 ± 0.1 | 2.00 ± 0.25 | 0.20 |
| *Gelatine:DATEM* | 10:0 | 2.6 ± 1.0 | 7.41 ± 0.94 | 0.20 |
| *clustered samples* | | | | |
| *Gelatine:DATEM* | 2:8 | 7.5 ± 1.5 | 8.91 ± 1.37 | 0.31 |
| *Gelatine:DATEM* | 5:5 | 37.6 ± 2.9 | 77.70 ± 20.46 | 0.53 |
| *non-clustered emulsions* | | | | |
| *Gelatine:WPI* | 0:10 | 2.1 ± 0.8 | 1.43 ± 0.04 | 0.20 |
| *Gelatine:WPI* | 10:0 | 1.9 ± 0.5 | 4.30 ± 0.72 | 0.20 |
| *clustered emulsions* | | | | |
| *Gelatine:WPI* | 2:8 | 12.3 ± 1.1 | 6.55 ± 0.69 | 0.33 |
| *Gelatine:WPI* | 5:5 | 47.7 ± 2.4 | 66.37 ± 15.67 | 0.55 |

We observed that clustering of WPI-stabilised emulsions with proanthocyanidins and hetero-aggregation of oppositely-charged emulsions allowed to control clustering oil droplets in o/w emulsions in a large size range. In Table 2, it can be seen that cluster size could be varied between a few microns to 50 μm for hetero-aggregation and up to 150 μm for clustering with GSE. Cluster size and the resulting effective volume fraction were shown to depend on the approach used. Figure 11 shows the effective volume fraction as a function of cluster size for the two clustering methodologies.
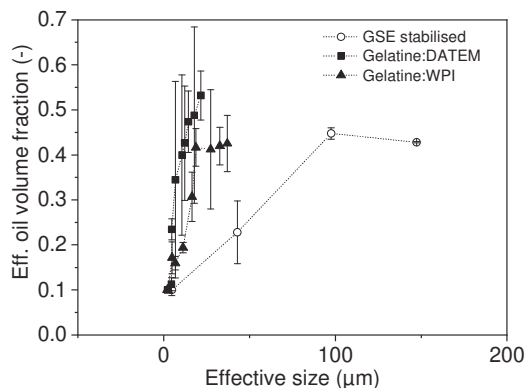
**Figure 11. Effective volume fraction of o/w emulsions as a function of the degree of oil droplet clustering for the different clustering methodologies: hetero-aggregation (full symbols: squares are gelatine-DATEM hetero-aggregated o/w emulsions, triangles are gelatine-WPI hetero-aggregated o/w emulsions) and open circles are WPI-stabilised o/w emulsions cross-linked with grape seed extract (proanthocyanidin). Absolute oil volume fraction is 0.10 (oil concentration 10%) for all emulsions. Error bars are standard deviations (n=3), and dotted lines are added to guide the eye.**

For the same cluster size, the effective oil volume fraction was smaller for the emulsions cross-linked with grape seed extract (proanthocyanidins) than for the hetero-aggregated o/w emulsions. We suggest that the effective oil volume fraction is related, to a certain extent, to the interaction strength of the clusters. The interaction strength within the clusters was shown to depend on the interactions responsible for the clustering. Large clusters prepared with grape-seed-extract (75% GSE/protein) showed a critical strain of 6.43±1.30%. In contrast, the largest clusters (about 50 μm) of strongly bound gelatine-DATEM emulsions showed a lower critical strain of about 4.0±0.8%. The multiple interactions in the grape seed extract stabilised droplet clusters lead to stronger clusters than the electrostatic interactions in the hetero-aggregates. Therefore, the packing of the oil droplets within the cluster and the ability to entrap the continuous phase are affected; stronger interactions cause a closer droplet packing and less water will be entrapped, leading to a lower effective volume fraction. Accordingly, the effective oil volume fraction for loosely clustered emulsions (hetero-aggregation) is higher. The differences in effective oil volume fraction between the two sets of hetero-aggregated emulsions are not discussed here due to rather large standard deviations of the measurements. Because of the clustering and the increase in effective volume fraction, for both methodologies, the viscosity increased. In Table 2, the viscosities at a shear rate of 50/s are shown. As expected, viscosity increases with increasing clustering of the droplets. Based on these results, we conclude that the presented methods of clustering allow not only control in cluster size but also control in interaction strength and cluster morphology, which provides a tool to alter the rheological behaviour of the resulting emulsions.

## 2.4 Conclusions

This study aimed to develop knowledge of how to manipulate both oil droplet cluster size, thus oil droplet inhomogeneity, and cluster strength in liquid o/w emulsions. Clustering by protein-proanthocyanidin

interactions and by hetero-aggregation of oppositely-charged emulsion droplets were shown to be effective methods to cluster oil droplets, as both methods induced controllable clustering in a cluster size range of 10 µm to 150 µm. Cluster size and strength were shown to be dominated by the interactions between the oil droplets. Strong interactions due to proanthocyanidin led to larger cluster sizes and higher cluster strength than weaker electrostatic interactions in the hetero-aggregated clusters. The clustering of the oil droplets led to an increase in viscosity by up to three orders of magnitude because of an increase in effective oil volume fraction. The effective volume fraction was shown to depend on cluster size, cluster strength, and cluster morphology. Stronger interactions lead to more compact clusters and a smaller increase in effective volume fraction. By manipulating the spatial distribution of oil droplets by droplet clustering, the extent of droplet heterogeneity and the interactions within and between droplet clusters showed to be highly effective in steering the rheological behaviour of the studied emulsions.

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# Chapter 3 - Clustering of oil droplets in o/w emulsions enhances perception of oil-related sensory attributes

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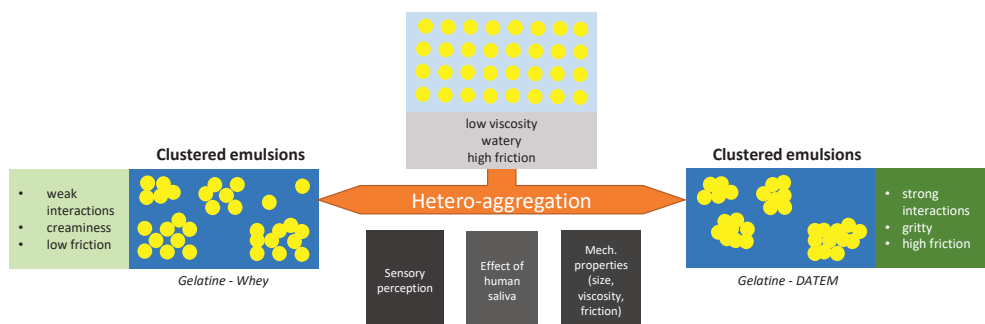
## Abstract

The sensory perception of o/w emulsions is determined by their structure and physicochemical properties of the emulsion. The aims of this study were (a) to determine the influence of oil droplet clustering in o/w emulsions on sensory perception and (b) to link their sensory attributes to rheological, tribological and structural properties. Clustered emulsions were prepared by combining o/w emulsions stabilised by different sets of emulsifiers: (a) positively-charged gelatine and negatively-charged whey protein (WPI), and (b) positively-charged gelatine and negatively-charged diacetyl tartaric acid ester of mono- and diglycerides (DATEM). Oil droplet clusters ranging in diameter from 1-50 μm were obtained. The difference in charge density between gelatine- and DATEM-stabilised oil droplets was higher than that between gelatine- and WPI-stabilised droplets. This difference allowed to alter the interaction strength within oil droplet clusters. The sensory perception of clustered emulsions was quantified using the Rate-All-That-Apply (RATA) methodology with untrained subjects (n=83). Participants assessed o/w emulsions varying in cluster size (1 μm to 50 μm), cluster strength (tuned by changing the emulsifier-pairs), and non-clustered emulsions with and without adjusted viscosity, as well as a reference emulsion with large single droplets (comparable in size to emulsions with large clusters). Creaminess and thickness intensities were significantly higher for clustered o/w emulsions compared to that of non-clustered o/w emulsions with the same oil content and similar oil droplet/cluster size. With increasing cluster size, creaminess and thickness intensities increased significantly for hetero-aggregated clusters with weak interactions (gelatine-whey protein). When cluster interactions were stronger (gelatine-DATEM), creaminess intensity increased to a lesser extent, and grittiness intensity increased considerably. Thickness and creaminess were strongly correlated to the rheological (*e.g.* consistency) and tribological properties (*e.g.* fiction coefficient at 10 mm/s) of o/w emulsions with clustered oil droplets. Grittiness and fattiness were strongly correlated to the tribological properties (slope of the mixed regime) of o/w emulsions and their interactions with saliva. We conclude that clustering of oil droplets in o/w emulsions by hetero-aggregation allows enhancing the sensory perception of fat-related attributes by tuning rheological and tribological properties and provides an effective method to structure liquid foods to obtain specific sensory properties.

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## 3.1 Introduction

Reduction of fat content in foods while maintaining desired sensory properties, such as creaminess, remains a challenge. The sensory perception of o/w emulsions is largely determined by the rheological and tribological properties, which are influenced by oil volume fraction and oil droplet size. With increasing oil volume fraction, the viscosity of o/w emulsions increases, and the perception of fat-related sensory attributes is enhanced (Chojnicka, Sala, de Kruif, & van de Velde, 2009; Lett, Norton, & Yeomans, 2016). Not only the oil volume fraction but also the oil droplet size determines the sensory properties of emulsions. Decreasing the oil droplet size at constant oil volume fraction has been shown to increase creaminess (Lett et al., 2016), possibly because of an increase in viscosity (Pal, 2011). The sensory perception of o/w emulsions is also related to several other physical characteristics such as flow behaviour and lubrication properties (Kim, Gohtani, & Yamano, 1996; Lett et al., 2016; Starov & Zhdanov, 2003). The rheological properties of o/w emulsions are mostly related to thickness and creaminess perception. In contrast, the lubrication properties are related to attributes such as melting, fattiness and creaminess (Akhtar, Stenzel, Murray, & Dickinson, 2005; de Wijk, Janssen, & Prinz, 2011; Izutsu & Wani, 1985).

Besides oil volume fraction and oil droplet size, the clustering of oil droplets in o/w emulsions has been demonstrated to affect the rheological properties of emulsions (Dalgleish, 2006). Clustering can be the result of different attractive interactions between oil droplets. Several studies have shown that oil droplets in o/w emulsions can be clustered by use of electrostatic interactions, also referred to as hetero-aggregation (Fuhrmann, Sala, Stieger, & Scholten, 2019a, Mao & McClements, 2011, 2012a, b, 2013a, b). Hetero-aggregation allows clustering of oil droplets in o/w emulsions in a controlled manner. To obtain hetero-aggregated oil droplets, two emulsions stabilised with oppositely charged emulsifiers are combined. Due to electrostatic attraction between the emulsifier-stabilised oil droplets, clusters are formed when repulsive interactions are overcome. Several emulsifier combinations for hetero-aggregates have been described, including ß-lactoglobulin and lactoferrin (Iqbal, Hameed, Baloch, & McClements, 2013; Mao & McClements, 2011, 2012a, b), whey protein isolate and modified starch (Mao & McClements, 2013b), and whey protein isolate and saponins (Maier, Oechsle, & Weiss, 2015; Maier, Zeeb, & Weiss, 2014). Recently, it was shown that cluster size and cluster strength of hetero-aggregated oil droplets in o/w emulsions could be controlled using gelatine in combination with whey protein or DATEM (Fuhrmann et al., 2019a). Several studies have demonstrated that emulsion viscosity increases largely as a result of oil droplet clustering (Mao & McClements, 2012a, b, 2013a). The main reason for the increase in viscosity by clustering of oil droplets is an increase in the effective volume fraction of the dispersed oil droplets (Tadros, 2004; Tadros, 1996). When oil droplets are clustered, water is enclosed within the clusters, which effectively increases the volume fraction of the dispersed phase. Such an effect may be compared to the incorporation of water droplets into w/o/w emulsions, where water droplets are emulsified and dispersed in the oil phase and thereby the volume fraction of the oil phase increases. For w/o/w emulsions, the increased volume fraction of the dispersed phase has a positive influence on sensory perception of fat-related attributes (Oppermann, Piqueras-Fiszman, de Graaf, Scholten, & Stieger, 2016; Oppermann, Renssen, Schuch, Stieger, & Scholten, 2015; Oppermann, Verkaaik, Stieger, & Scholten, 2017). Double emulsions can, therefore, be used as a strategy for fat reduction while maintaining the sensory perception of fat-related attributes such as creaminess.

Structural changes of emulsions occurring during oral processing can influence sensory perception, especially the perception of attributes perceived at later stages of the consumption process (Vingerhoeds, Blijdenstein, Zoet, & van Aken, 2005). During oral processing, saliva is incorporated into the emulsion, which introduces salivary proteins, enzymes and salts. Interactions between saliva and emulsion droplets can lead to changes in the emulsion structure. O/w emulsions stabilised with whey protein isolate have been observed to flocculate upon mixing with saliva due to electrostatic interactions between positively-charged whey proteins and negatively-charged mucins (Chojnicka et al., 2009; Scholten, 2017; Silletti, Vingerhoeds, Norde, & van Aken, 2007). These structural changes during oral processing influence both rheological and tribological properties of the emulsions and can influence sensory properties.

To the best of our knowledge, the sensory perception and tribological properties of o/w emulsions with clustered oil droplets have not been studied yet. The aims of this study were (a) to determine the influence of oil droplet clustering in o/w emulsions on sensory perception and (b) to link their sensory attributes to rheological, tribological and structural properties. We hypothesised that creaminess and thickness perception of o/w emulsions is enhanced by clustering of oil droplets and that the degree of enhancement is related to cluster size and strength. Additionally, we hypothesised that the interactions between saliva and hetero-aggregated oil droplets influence the structure and sensory properties of clustered o/w emulsions by interfering with the electrostatic interactions between oil droplets.

## 3.2. Materials and methods

### 3.2.1 Materials

Whey protein isolate (BiPRO WPI) was obtained from Davisco (Lot # JE 062-3-420, USA). Diacetyl tartaric acid ester of mono- and diglycerides (DATEM) was kindly provided by CP Kelco (USA). Gelatine Type 250 PS 30 was obtained from Rousselot (Lot #1207647, The Netherlands). Citric acid (p.a.) and sodium hydroxide (p.a.) were obtained from Sigma Aldrich (Steinheim, Germany). Sunflower oil (Reddy, The Netherlands), vanilla aroma (Dr Oetker, Germany) and sugar (Giro, The Netherlands) were purchased from a local retailer. All ingredients used were food grade.

### 3.2.2 Preparation of o/w emulsions

The composition of all studied o/w emulsions together with droplet size, critical strain and zeta potential is shown in Table 1, and a schematic overview of o/w emulsions can be found in Figure 1. Further information to flow behaviour and tribological properties can be found in Table 2 a. O/w emulsions with clustered oil droplets were prepared using hetero-aggregation by combining oppositely-charged o/w emulsions. Emulsions differed mainly in cluster size (small, medium, large) and cluster strength (weakly and strongly interacting clusters). As a reference, o/w emulsions with individual oil droplets of the size of the large oil droplet clusters were prepared. Another reference sample consisted of an o/w emulsion with the same viscosity of the emulsion containing large oil droplet clusters. The viscosity of the reference emulsion was adjusted by addition of xanthan as a thickening agent. In total, 13 emulsions were prepared (Table 1)
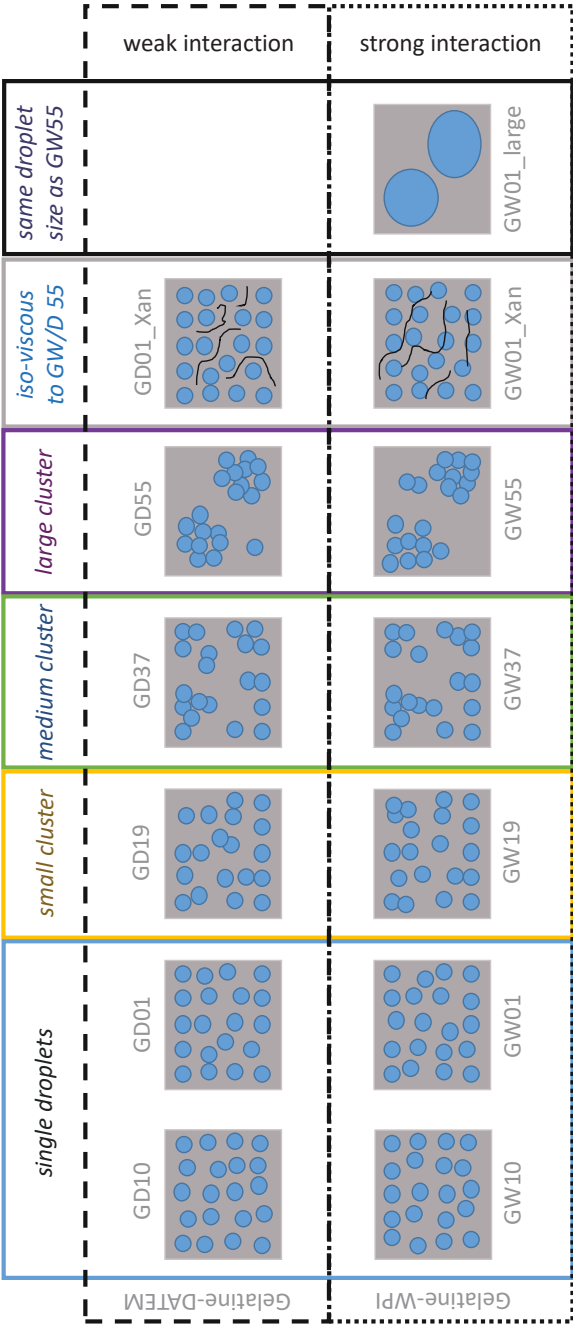
**Figure 1. Schematic overview of the o/w emulsions prepared. G, D and W indicate the emulsifiers used (gelatine, DATEM and whey protein isolate), Xan indicates the thickener used (xanthan). Numbers indicate mixing ratio of oppositely-charged o/w emulsions, *i.e.* GD55 refers to 50% gelatine-stabilised emulsion mixed with 50% DATEM-stabilised emulsion and GD10 refers to 100% gelatine and 0% DATEM (see Table 1 for composition).**

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Table 1. Composition of the o/w emulsions. All emulsions had an oil volume fraction of 0.2. The aqueous phase of all o/w emulsions contained 0.63% sugar (w/v), 0.018% (w/v) vanilla aroma and 7.5 mM citric acid/ NaOH solution. WPI: whey protein isolate, DATEM: diacetyl tartaric acid ester of mono- and diglycerides

| Samples | | Emulsion composition | | | | | Emulsion mixtures | | | Emulsion characteristics | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| o/w emulsion | Sample code | Gelatine | DATEM | WPI | Xanthan | pH | Gelatine stabilised emulsion | DATEM stabilised emulsion | WPI stabilised emulsion | Zeta Potential | Critical Strain | Size |
| | | % (w/v) | % (w/v) | % (w/v) | (% w/v) | | (%) | (%) | (%) | (mV) | (%) | (µm) |
| **DATEM non-clustered** | GD 01 | 0 | 0.12 | 0 | 0 | 5 | 0 | 100 | 0 | -60±1 | - | 1.9±0.6 |
| **Gelatine non-clustered (pH 5)** | GD 10 | 0.6 | 0 | 0 | 0 | 5 | 100 | 0 | 0 | +10±1 | - | 2.6±0.1 |
| **Gelatine-DATEM cluster (small)** | GD 19 | 0.06 | 0.11 | 0 | 0 | 5 | 10 | 90 | 0 | - | - | 5.6±0.6 |
| **Gelatine-DATEM cluster (medium)** | GD 37 | 0.18 | 0.08 | 0 | 0 | 5 | 30 | 70 | 0 | - | - | 10.8±0.9 |
| **Gelatine-DATEM cluster (large)** | GD 55 | 0.3 | 0.06 | 0 | 0 | 5 | 50 | 50 | 0 | -1±8 | 4.1±0.8 | 37.6±1.7 |
| **WPI non-clustered** | GW 01 | 0 | 0 | 0.19 | 0 | 7 | 0 | 0 | 100 | +7±2 | - | 2.1±0.5 |
| **Gelatine non-clustered (pH 7)** | GW 10 | 0.6 | 0 | 0 | 0 | 7 | 100 | 0 | 0 | -45±3 | - | 1.9±0.3 |
| **Gelatine-WPI cluster (small)** | GW 19 | 0.06 | 0 | 0.17 | 0 | 7 | 10 | 0 | 90 | - | - | 7.9±0.3 |
| **Gelatine-WPI cluster (medium)** | GW 37 | 0.18 | 0 | 0.13 | 0 | 7 | 30 | 0 | 70 | - | - | 14.8±0.7 |
| **Gelatine-WPI cluster (large)** | GW 55 | 0.3 | 0 | 0.1 | 0 | 7 | 50 | 0 | 50 | -9±2 | 1.8±0.6 | 47.7±1.4 |
| **Reference: Iso-viscous GD55** | GD01_Xan | 0 | 0.12 | 0 | 0.3 | 5 | 0 | 100 | 0 | - | - | 1.9±0.6 |
| **Reference: Iso-viscous GW55** | GW01_Xan | 0 | 0 | 0.19 | 0.3 | 7 | 0 | 0 | 100 | - | - | 2.1±0.5 |
| **Reference: Iso-size GW55** | GW01_large | 0 | 0 | 0.19 | 0 | 7 | 0 | 0 | 100 | - | - | 40.9±9.5 |

Preparation of non-clustered o/w emulsions

First, aqueous solutions of the different emulsifiers were prepared. Whey protein isolate (6.4 mg/mL aqueous solution) was dissolved in a 7.5 mM citric acid solution. The solution was stirred for two hours at room temperature, after which the pH was set to 7 with 1 M NaOH. For gelatine solutions, gelatine (20 mg/mL aqueous phase) was added to the citric acid solution and heated at 80°C for 30 min whilst stirring to hydrate and dissolve the gelatine. The pH was then adjusted to 5 or 7 using 1 M NaOH/1 M HCl. DATEM was dissolved in the oil phase (4 mg/mL oil) due to its limited solubility in water. The solution was heated at 80°C for 10 min. Both gelatine and DATEM solutions were cooled to 40°C before further processing.

To prepare non-clustered o/w emulsions, the aqueous phase and the oil phase were mixed slowly, while pre-emulsifying with a rotor-stator homogeniser (Ultra-Turrax T25, IKA, Germany) at 8000 rpm for 3 min. A stock emulsion with a total oil volume fraction of 0.4 was obtained. Subsequently, the pre-emulsions were homogenised in a 2-stage homogeniser (PandaPlus, Niro Soavi, Parma, Italy) at 50 bar and 250 bar for 2 cycles. The emulsions were diluted using the corresponding aqueous solution to obtain a final oil volume fraction of 0.2. Emulsions with larger droplet sizes (GW01_large) were emulsified with a rotor-stator homogeniser (Ultra-Turrax T25, IKA, Germany) at 8000 rpm for 5 min and not further processed. To obtain reference emulsions with adjusted viscosity (GW01_Xan and GD01_Xan), stock emulsions (volume fraction oil 0.4) were diluted with xanthan solutions (0.6% (w/v) in citric acid solution pH 5/7) to obtain a final xanthan concentration of 0.3% (w/v emulsion) and a final oil volume fraction of 0.2. After the preparation of emulsions, 0.63% (w/v) sugar and 0.018% (w/v) vanilla aroma were added to all emulsions. The emulsions were stored for a maximum of one week at 4°C before physical measurements were performed. The emulsions used for sensory tests were prepared 24 hrs before providing the samples to participants.

Preparation of o/w emulsions with clustered oil droplets

Hetero-aggregated o/w emulsions were prepared by combining two non-clustered o/w emulsions at different volume ratios (v/v). After combining the two non-clustered o/w emulsions, the hetero-aggregated o/w emulsions were slowly stirred and subsequently stored for 24 hrs at 4°C before further use. The pH of the emulsions did not change upon mixing. Upon storage for 3 days at room temperature, clustered emulsions with large clusters (GW55, GD55) and emulsions with large droplets (GW01_large) showed creaming behaviour. All other clustered emulsions and non-clustered emulsions did not display creaming. Creaming was reversible by careful re-dispersion by manually shaking. None of the non-clustered and clustered emulsions showed coalescence. The mixing ratios of the oppositely-charged non-clustered emulsions can be found in Table 1.

All samples were coded with a 2-letter-2-digit code. GD refers to emulsions stabilised with gelatine and/or DATEM, whereas GW refers to emulsions stabilised with gelatine and/or whey proteins. Numbers indicate the mixing ratio between the two oppositely-charged non-clustered emulsions. For example, the code GD55 refers to an emulsion consisting of 50% (w/v) gelatine-stabilised emulsion mixed with 50% (w/v) DATEM-stabilised emulsion. In comparison, GD01 denotes an emulsion consisting of 100% (w/v) DATEM-stabilised emulsion.

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### 3.2.3 Determination of oil droplet cluster size

Static light scattering (Mastersizer 2000S; Malvern Instruments, Ltd., Worceshire, UK) was used to quantify the particle size distribution of non-clustered o/w emulsions. The refractive index of the dispersed phase (sunflower oil) was set at 1.47 and that of the continuous water phase at 1.33. Three measurements were done per aliquot. Due to the shear forces applied during the measurement, the particle size distribution of o/w emulsions with clustered oil droplets could not be obtained with the Mastersizer, since oil droplet clusters broke up during the measurement.

For o/w emulsions with clustered oil droplets, the effective size was determined by light microscopy. The emulsions were diluted with an aqueous solution of the same pH to separate clusters on a microscope slide. The slide was covered with a coverslip. From each slide, 6 images in predefined positions were taken using an optical light microscope (Axioskop 2 plus, Carl Zeiss AG, Germany) equipped with a camera (Axiocam ERc 5S, Carl Zeiss AG, Germany) and Visio imaging software. The images were analysed for particle area using ImageJ/Fiji. From the particle area, an effective cluster diameter, referred to the as effective diameter, was calculated assuming a spherical shape for the clusters. The brightness was adjusted automatically. A threshold was used to define a minimum size of 0.1 µm to remove background noise.

### 3.2.4 Zeta potential

The zeta potential was determined using a Zetasizer Nano ZS series (Malvern Instruments, Worcestershire, UK). Single and clustered emulsions were diluted at least 100x with the corresponding aqueous phase (without emulsifier). Each emulsion was measured in triplicate at 20°C.

### 3.2.5 Rheological characterisation of o/w emulsions

Rheological tests were conducted with an Anton Paar 302 Rheometer (MCR 302, Anton Paar GmbH, Graz, Austria) using a concentric cylinder cup geometry (sandblasted CC17). Gap size was set at 0.02 mm. Flow curves were determined by measuring viscosity at an increasing shear rate from 1-100 s-1 in a time period of 10 min at 22°C. Measurements were performed in triplicates with newly prepared emulsions to obtain averaged values. Flow curves were fitted in a shear rate range from 1-100 s-1 to the Ostwald-de Waele power-law model (Ostwald, 1925):

$$\eta = K * \dot{\gamma}^{n-1} \qquad\qquad 1$$

In this model, η represents viscosity (Pa·s), $\dot{\gamma}$ (s-1) shear rate, K flow consistency index (Pa·s$^n$) and n flow index, which indicates the magnitude of the shear-thinning behaviour (0 < n < 1). Additionally, the apparent viscosity at a shear rate of 50 s-1 is reported.

To estimate the interaction strength of clustered emulsions, the critical strain and the stress at the critical strain were determined. To obtain the critical strain and the stress at critical strain, oscillatory tests were performed using a parallel plate geometry with a diameter of 50 mm. The gap height was set to 1 mm. A strain sweep was conducted at a frequency of 10 rad/s (1.59 Hz) and strain increased from 0.01% to 100% at 22°C. The storage modulus G' and the loss modulus G'' were determined. Critical strain and stress at critical strain were defined as the strain and the stress at which the storage modulus G' deviated by 5% from the values found in the linear viscoelastic regime.

### 3.2.6 Tribological characterisation of o/w emulsions

An Anton Paar Rheometer (MCR 302, Anton Paar GmbH, Graz, Austria) equipped with a tribological setup (T-PTD 200-SN81766963) was used to measure tribological properties. The selected geometry was a glass ball-on-three PDMS-pins setup (PDMS pins (r=2.8 mm), glass ball (d=12.6 mm)). 600 μL of the emulsion was loaded into the cell, and a normal force of 1 N was applied. The rotational speed was increased from 0.1 rpm to 1000 rpm (equivalent to 0.47 mm/s to 470 mm/s) for run 1 and 3 and was decreased from 1000 rpm to 0.1 rpm for run 2 and 4 in 300 s each. Torque and normal force were recorded and friction coefficients, μ, calculated. The temperature was kept at 22°C. All measurements were performed in triplicate with new batches. Between each set of measurement, the pins were renewed to avoid wear of the tribo-pair. During the first run, the PDMS pins were still adjusting to their final position, and the results were not taken into account. Only the third run was used for further analyses, in which the speed was increased over time. From 3 individual measurements of the 3rd run, the friction coefficient, μ, was taken as a function of speed. An overview of frictional parameters extracted from the Stribeck curves is depicted in Figure 2.
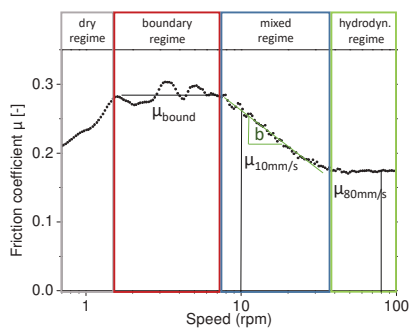


**Figure 2. Friction coefficient as a function of speed for an o/w emulsion with an illustration of the extracted tribological parameters: average friction coefficient in the boundary regime ($\mu_{bound}$), friction coefficients at 10 mm/s ($\mu_{10mm/s}$) and 80 mm/s ($\mu_{80mm/s}$) and exponent b (slope of the curve in mixed lubrication regime).**

Three friction coefficients were extracted from different sections of the curves: the average friction coefficient in the boundary regime ($\mu_{bound}$), and the friction coefficients at 10 mm/s ($\mu_{10mm/s}$) and 80 mm/s ($\mu_{80mm/s}$). Additionally, a power-law model was used to determine the exponent b, which was used to characterise changes in friction coefficient with speed in the mixed lubrication regime (slope of the curve in mixed regime) as

$$\mu \sim speed^b \qquad \qquad 2$$

### 3.2.7 Saliva addition to o/w emulsions

To partly mimic in-mouth conditions, human saliva was added to the emulsions. Human saliva was collected from 10 participants and pooled together as described in literature (Silletti et al., 2007). Saliva was collected in cooled plastic tubes (Greiner centrifuge tubes 15 mL, Merck, US) from volunteers (n=10), after rinsing their mouth with water. Saliva was centrifuged at 10000 g for 30 min at cooled conditions to remove debris and reduce air content (Beckmann, Avanti TM J-25 I, JA-21, Beckman Coulter B.V. Mijdrecht, The Netherlands). The supernatant was collected. Emulsions and saliva were combined in a 1:1 ratio, based

on the ratio commonly used in literature (Laguna, Farrell, Bryant, Morina, & Sarkar, 2017; Vingerhoeds et al., 2005), in an Eppendorf tube and carefully mixed for 1 minute by repeatedly turning the tube upside down. The temperature during mixing was not controlled. The pH after mixing was not further adjusted. To characterise the effect of saliva addition on the physical properties of emulsions, particle and cluster size, rheological and tribological properties were determined as described above.

### 3.2.8 Sensory evaluation of o/w emulsions

Thirteen o/w emulsions (Figure 1) were evaluated by an untrained panel (n=83 participants; 62 female, 21 male; mean age 23.5±3.8 years, age range 19-38 years) using the Rate-All-That-Apply methodology (RATA) (Meyners, Jaeger, & Ares, 2016). Participants were recruited from the Wageningen University & Research campus. Participants were asked to confirm the absence of intolerance towards the ingredients present in the emulsions and received financial compensation upon completion of the study. Participants received a digital copy of the sensory terms and their explanations before participation. An overview of the attributes and explanations can be found in the Appendix. Subjects gave written informed consent before the study.

The emulsions were presented in two sessions of 60 min in randomised order. Twenty mL of each emulsion was served in yellow 25 mL plastic cups labelled with a random 3-digit code. Participants rated the emulsions on 20 attributes (Table 2). The attributes for the RATA, as well as the explanations, were selected from several previous studies using emulsions (Benjamins, Vingerhoeds, Zoet, de Hoog, & van Aken, 2009; Oppermann et al., 2017; van Aken, Vingerhoeds, & de Wijk, 2011). The RATA assessment was performed in two parts. First, "mouth-feel" attributes were assessed. Subjects were instructed to spit out the emulsions at the moment they would usually swallow them. After spitting out the emulsions, attributes were selected and scored. Then, participants evaluated the "after-feel" attributes. Subjects were instructed to take a sip of the emulsion and swallow, wait for 10 s and then assess the attributes. The time was indicated using a stopwatch integrated into the digital questionnaire. Between tasting of two samples, participants had a 2 min break and were asked to rinse their mouth with water and eat white bread. Intensity ratings were done using a 9-point scale with anchors "weak" and "strong" and the possibility to choose "not applicable". Data was collected using tablets with a questionnaire made in EyeQuestion (Version 4.11.3).

### 3.2.9 Statistical data analysis

Statistical analysis of the RATA data was done following the procedure described previously (Ares et al., 2014; Meyners et al., 2016; Oppermann et al., 2017). RATA data were considered as continuous intensity scores with "not applicable" being evaluated as 0 (Meyners et al., 2016). A two-way ANOVA was carried out. Significance levels were described using Tukey's Honest Significant Difference Test (HSD) at 95% confidence level. Significance levels for physical parameters (cluster size, friction coefficients) were calculated similarly. Principal component analysis, correlation analysis based on a Spearman's rank correlation and cluster analysis (k-means) were performed. For these calculations, R Studio (Version 1.0.143) was used with the additional packages SensoMineR, factoextra, corrplot, multcomp, ggplot2, multcompView and agricolae and FactoMineR.

## 3.3. Results and discussion

### 3.3.1 Emulsion characteristics

Table 2 gives an overview of effective oil droplet cluster size, rheological and tribological properties of all o/w emulsions with (b) and without (a) added saliva. The effect of saliva addition on effective oil droplet cluster size, rheological and tribological properties of the o/w emulsions is discussed in section 3.3.3. Hetero-aggregated emulsions varied in cluster size from 10 μm (small) to 50 μm (large). As expected, cluster size was controlled by the mixing ratio of oppositely charged single o/w emulsions. The largest cluster size was obtained by mixing equal amounts of oppositely charged single emulsions (GD55 and GW55). Cluster size was found to be around 47 μm for GW55 and about 40 μm for GD55. Smaller clusters were formed for unequal mixing ratios of oppositely charged emulsions (GD19, GD37, GW19 and GW37), as an excess in droplets of one charge limits cluster growth (Fuhrmann et al., 2019a). All non-clustered emulsions (GD01, GD10, GW01 and GW10) including emulsions with an adjusted viscosity (GW01_Xan and GD01_Xan), had oil droplet sizes around 1-2 μm, except for GW01_large, which had an oil droplet size of 41 μm. In Figure 3, we show exemplarily micrographs of non-clustered and clustered emulsions.
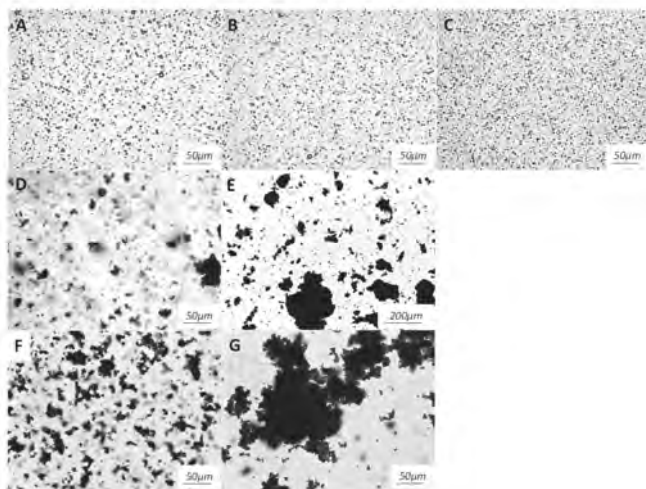


**Figure 3. Micrographs of non-clustered emulsions varying in emulsifier (A: gelatine at pH 7, B: WPI, C: DATEM) and clustered emulsions (D: GD small, E: GD large, F:GW small, G:GW large). Scale bars show for images A-D and F-G 50 μm, for image E 200 μm.**

Typical flow curves of the non-clustered and clustered o/w emulsions together with the fits of the Ostwald-de Waele power-law model are shown in Figure 4.

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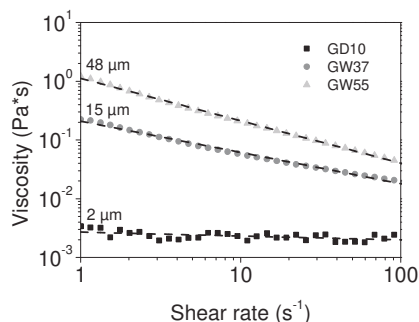


**Figure 4. Typical flow curves of non-clustered o/w emulsion (GD10), hetero-aggregated clustered o/w emulsions with medium cluster size (GW37) and hetero-aggregated clustered o/w emulsions with large cluster size (GW55) as a function of shear rate in a range from 1 to 100 s$^{-1}$. Dashed lines show the best fits to the Ostwald-de Waele power-law model. The total oil volume fraction was 0.2 for all emulsions.**

Most non-clustered emulsions (GD01, GD10, GW10 and GW01, GW01_large) showed flow indices n of around 1, thus exhibited Newtonian flow behaviour. Non-clustered gelatine-stabilised emulsions at pH 5 (GD10) showed weak shear-thinning behaviour (n=0.86). Highly clustered emulsions displayed flow index values 0<n<0.21, indicating strong shear-thinning behaviour. The effect of droplet clustering was also seen in the consistency, K. For non-clustered emulsions (GD10, GD01, GW01, GW10), K was in the range of 0.001 to 0.01 Pa·s$^n$. For emulsions with large clusters (GD55, GW55), K was 1.0 and 2.8 Pa·s$^n$. K increased by three orders of magnitude for these clustered emulsions compared to that of the non-clustered emulsions, which is mainly related to the increase in effective volume fraction caused by oil droplet clustering. As mentioned above, clustered emulsions entrap water between droplets, which causes a decrease in free aqueous bulk phase (Mao & McClements, 2013a; van Aken, Oliver, & Scholten, 2015b). These findings are in line with the results shown in the studies by Mao and co-workers (2012a, b, 2013a). The viscosity values of hetero-aggregated emulsions in our study were higher than those reported for hetero-aggregated clustered emulsions consisting of modified starch-/whey-stabilised clusters (Mao & McClements, 2013a), but comparable to those of clustered emulsions stabilised by proteins (Mao & McClements, 2012a).

Also, the interaction strength of clustered emulsions was assessed by determining the critical strain upon oscillatory deformation (Table 1). The critical strain of non-clustered emulsions and smaller clusters could not be determined, as the loss modulus of emulsions with small clusters was higher than the storage modulus. The critical strain was around 2% for GW55 and 4% for GD55. The stronger electrostatic attraction within clusters stabilised with gelatine and DATEM (GD) thus led to a 2x higher critical strain compared to clusters with gelatine and WPI (GW) due to differences in charge density of the emulsifiers. The assessment of the zeta-potential (Table 1) showed that DATEM-stabilised emulsions were, as expected, negatively charged, with a zeta potential of around -60 mV. WPI-stabilised emulsions displayed a negative charge of -45 mV at pH 7. Gelatine (pI around 8-9) showed a slightly positive charge of +10 mV at pH 5 and of +7 mV at pH 7. The difference in zeta potential for gelatine-DATEM emulsions at a pH of 5 was, therefore, around 70 mV and for gelatine-WPI emulsions around 50 mV, at pH 7. The gelatine-DATEM combination, hence, provided stronger electrostatic attraction between oil droplets, and higher cluster strength. Table 2 (a) gives an overview of the tribological properties of all o/w emulsions. The friction

coefficient of non-clustered emulsions stabilised by DATEM was lower than that of non-clustered emulsions stabilised by gelatine. As the oil droplet size was similar for both emulsions, the difference in friction coefficient was related to the different emulsifiers used. Due to the higher hydrophobicity of DATEM compared to that of gelatine, oil droplets stabilised with DATEM had a higher affinity for the hydrophobic PDMS surface, thus could form a film on the surface more easily and consequently improve lubrication. Furthermore, DATEM-stabilised droplets coalesced more than gelatine-stabilised droplets as observed by the occurrence of oil patches on the emulsion surface after the tribological measurement (data not shown). The thickened emulsion (GD01_Xan) showed improved lubrication properties ($\mu_{bound}$, $\mu_{10mm/s}$ and $\mu_{80mm/s}$), with friction coefficients between 0.11 and 0.24, compared to non-clustered emulsions stabilised with DATEM (GD01), with friction coefficients between 0.17 and 0.31. This is in line with observations made by Chojnicka-Paszun & de Jongh (2014), who suggested that xanthan might mask surface roughness and improve lubrication. Clustered emulsions prepared with gelatine and DATEM (GD55) showed a relatively high friction coefficient of $\mu_{bound}$=0.37 in the boundary regime, a sharp drop at intermediate speeds ($\mu_{10mm/s}$ = 0.17) and an increase in the friction coefficient at higher speeds ($\mu_{10mm/s}$ = 0.20). In the low-speed regime, the lubrication behaviour of the emulsions with strongly bound clusters (GD55) was dominated by the tribological properties of the gelatine emulsion. With increasing speed, the friction coefficient of clustered emulsions (GD55) decreased more strongly than that of homogeneous gelatine-stabilised emulsions (GD10). This behaviour might possibly be related to a higher affinity to coalescence and film formation of DATEM-stabilised droplets, as the emulsion droplets are closer to each other within clustered samples than in non-clustered samples. For large clusters consisting of gelatine and WPI (GW55), all friction parameters ($\mu_{bound}$, $\mu_{10mm/s}$ and $\mu_{80mm/s}$) were below the values of non-clustered emulsions. $\mu_{10mm/s}$ decreased from above 0.3 for non-clustered emulsions (GW01 and GW10) to 0.19 for clustered emulsions. By clustering, the effective volume fraction of oil droplets increased, which seemed to improve the lubrication properties of the emulsions. Clustering decreases the distance between droplets in the emulsion, and as such, coalescence and accompanying film formation might occur more easily. Higher interaction strength might also explain the higher $\mu_{bound}$, observed for large GD clusters (GD55), compared to weakly bound clusters (GW55). Higher interaction strength between the droplets increases the stiffness of the clusters. Less deformable, thus stiffer clusters may spread less easily on the surface of the tribo-pair. This effect of droplet stiffness has been previously observed in double w/o/w emulsions (Oppermann et al., 2017). Homogeneous emulsions with large droplets (GW01_large; $\mu_{10mm/s}$ = 0.43) had higher friction coefficients than homogeneous emulsions with smaller droplets (GW01; $\mu_{10mm/s}$ = 0.35 ). This is in agreement with previous studies on non-clustered o/w emulsions with varying droplet size (Dresselhuis et al., 2007).

To summarise, the viscosity of hetero-aggregated clustered o/w emulsions strongly increased compared to that of non-clustered o/w emulsions with the same oil volume fraction due to an increase in effective volume fraction. The friction coefficient of hetero-aggregated clustered o/w emulsions with strongly interacting clusters was higher in the boundary and the beginning of the mixed regime than that of emulsions with weakly interacting clusters. The friction coefficients in the boundary regime ($\mu_{bound}$) and at higher speeds ($\mu_{80mm/s}$) depended on the emulsifier type, and thus cluster strength.

**3**

Table 2a. Physical properties of the o/w emulsions (oil volume fraction 0.2). For oil droplet size and friction properties, values with the same superscript letter (Tukey-HSD test) are not significantly different at p<0.05. F and p-values provided indicate the variation within one variable. Indications of significant differences between variables before and after addition of saliva are given as (ns)(p>0.05), *(p<0.05), **(p<0.01) or ***(p<0.001) .

| o/w emulsion | Sample code | Effective cluster size (µm) | Consistency K (Pa·s^n) | Flow index n (-) | $\mu_{bound}$ (-) | $\mu_{10mm/s}$ (-) | $\mu_{80\,mm/s}$ (-) | b exponent (/mm/s) |
|---|---|---|---|---|---|---|---|---|
| | | $F=149.5$ $p<0.001$ | | | $F=40.3$ $p<0.001$ | $F=2.3$ $p>0.05$ | $F=10.8$ $p<0.001$ | |
| **Non-clustered emulsions** | | | | | | | | |
| **WPI** | GW 01 | 2.1±0.5[a] | 0.002 | 0.92 | 0.31±0.01[ade] | 0.35±0.04[a] | 0.23±0.02[bc] | -0.34 |
| **Gelatine (pH 7)** | GW 10 | 1.9±0.3[a] | 0.005 | 0.96 | 0.41±0.02[b] | 0.31±0.04[a] | 0.24±0.05[bc] | -0.20 |
| **Equi-viscous control (to GW55)** | GW01_Xan | 2.1±0.5[a] | 1.680 | 0.22 | 0.36±0.02[bde] | 0.26±0.01[a] | 0.17±0.01[acd] | -0.24 |
| **Equi-sized control (to GW55)** | GW01_large | 40.9±9.5[de] | 0.003 | 0.92 | 0.43±0.01[b] | 0.43±0.09[a] | 0.22±0.05[bcd] | -0.41 |
| **DATEM** | GD 01 | 1.9±0.6[a] | 0.002 | 0.94 | 0.31±0.01[ade] | 0.25±0.04[a] | 0.17±0.01[acd] | -0.16 |
| **Gelatine (pH 5)** | GD 10 | 2.6±0.1[a] | 0.010 | 0.88 | 0.41±0.03[b] | 0.32±0.04[a] | 0.26±0.02[b] | -0.25 |
| **Equi-viscous control (to GD55)** | GD01_Xan | 1.9±0.6[a] | 2.004 | 0.22 | 0.24±0.01[a] | 0.20±0.01[a] | 0.11±0.01[a] | -0.17 |
| **Gelatine-DATEM clustered emulsions ("strong clusters")** | | | | | | | | |
| **Small cluster** | GD 19 | 5.6±0.6[ab] | 0.033 | 0.56 | | | | |
| **Medium cluster** | GD 37 | 10.8±0.9[bc] | 1.310 | 0.07 | 0.56±0.04[c] | 0.25±0.25[a] | 0.12±0.01[a] | -0.55 |
| **Large cluster** | GD 55 | 37.6±1.7[d] | 2.800 | 0.07 | 0.37±0.04[bd] | 0.17±0.01[a] | 0.20±0.01[bcd] | -0.54 |
| **Gelatine-WPI clustered emulsions ("weak clusters")** | | | | | | | | |
| **Small cluster** | GW 19 | 7.9±0.3[ab] | 0.013 | 0.69 | | | | |
| **Medium cluster** | GW 37 | 14.8±0.7[c] | 0.178 | 0.51 | 0.29±0.03[ae] | 0.22±0.10[a] | 0.15±0.03[ad] | -0.54 |
| **Large cluster** | GW 55 | 47.7±1.4[e] | 1.000 | 0.31 | 0.28±0.02[a] | 0.19±0.01[a] | 0.17±0.01[acd] | -0.27 |

Table 2b. Physical properties of the o/w emulsions (oil volume fraction 0.2) after addition of saliva. Addition of saliva refers to o/w emulsions mixed with saliva in a 1:1 ratio. For oil droplet size and friction properties, values with the same superscript letter (Tukey-HSD test) are not significantly different at p<0.05. F and p-values provided indicate the variation within one variable. Indications of significant differences between variables before and after addition of saliva are given as (ns)(p>0.05), *(p<0.05), **(p<0.01) or ***(p<0.001).

| o/w emulsion | Sample code | Effective cluster size ($\mu$m) | Consistency $K$ (Pa·s$^n$) | Flow index $N$ (-) | $\mu_{bound}$ (-) | $\mu_{10\,mm/s}$ (-) | $\mu_{80\,mm/s}$ (-) | b exponent (/mm/s) |
|---|---|---|---|---|---|---|---|---|
| | | $F=33.8$ $p<0.01$ | | | $F=10.6$ $p<0.001$ | $F=2.1$ $p>0.05$ | $F=4.7$ $p<0.01$ | |
| **Non-clustered emulsions** | | | | | | | | |
| WPI | GW 01 | $1.3\pm0.3^{b*}$ | 0.01 | 0.99 | $0.41\pm0.01^{bcde**}$ | $0.38\pm0.07^{a(ns)}$ | $0.27\pm0.07^{ab(ns)}$ | -0.16 |
| Gelatine (pH 7) | GW 10 | $25.1\pm9.9^{cd**}$ | 0.251 | 0.38 | $0.31\pm0.01^{a**}$ | $0.30\pm0.04^{a(ns)}$ | $0.22\pm0.02^{ab(ns)}$ | -0.14 |
| Equi-viscous control (to GW55) | GW01_Xan | $2.5\pm0.6^{ab(ns)}$ | 0.26 | 0.43 | $0.34\pm0.02^{ac(ns)}$ | $0.34\pm0.02^{a*}$ | $0.19\pm0.03^{b(ns)}$ | -0.21 |
| Equi-sized control (to GW55) | GW01_large | $48.7\pm8.4^{e(ns)}$ | 0.003 | 0.94 | $0.34\pm0.01^{ac**}$ | $0.35\pm0.17^{a(ns)}$ | $0.23\pm0.10^{ab(ns)}$ | -0.56 |
| DATEM | GD 01 | $1.2\pm0.1^{b(ns)}$ | 0.18 | 0.7 | $0.31\pm0.01^{a(ns)}$ | $0.31\pm0.05^{a(ns)}$ | $0.24\pm0.03^{ab*}$ | -0.13 |
| Gelatine (pH 5) | GD 10 | $25.7\pm8.2^{cd**}$ | 0.173 | 0.42 | $0.35\pm0.02^{abc*}$ | $0.35\pm0.04^{a(ns)}$ | $0.24\pm0.01^{ab(ns)}$ | -0.19 |
| Equi-viscous control (to GD55) | GD01_Xan | $2.0\pm0.4^{ab**}$ | 0.25 | 0.44 | $0.37\pm0.01^{abcd**}$ | $0.37\pm0.08^{a*}$ | $0.23\pm0.04^{ab**}$ | -0.43 |
| **Gelatine-DATEM clustered emulsions** | | | | | | | | |
| Small cluster | GD 19 | $5.6\pm2.8^{ab(ns)}$ | 0.03 | 0.48 | $0.45\pm0.07^{de(ns)}$ | $0.43\pm0.02^{a(ns)}$ | $0.39\pm0.01^{a**}$ | -0.7 |
| Medium cluster | GD 37 | $14.5\pm3.9^{ac(ns)}$ | 0.09 | 0.43 | $0.44\pm0.03^{bde*}$ | $0.43\pm0.13^{a*}$ | $0.36\pm0.01^{ab***}$ | -0.61 |
| Large cluster | GD 55 | $36.3\pm7.5^{de(ns)}$ | 0.09 | 0.44 | | | | |
| **Gelatine-WPI clustered emulsions** | | | | | | | | |
| Small cluster | GW 19 | $7.5\pm2.8^{ab(ns)}$ | 0.022 | 0.5 | $0.37\pm0.02^{abcd*}$ | $0.50\pm0.15^{a*}$ | $0.40\pm0.15^{a*}$ | -0.19 |
| Medium cluster | GW 37 | $7.5\pm2.4^{ab**}$ | 0.061 | 0.76 | $0.49\pm0.06^{e**}$ | $0.53\pm0.05^{a**}$ | $0.36\pm0.02^{ab**}$ | -0.82 |
| Large cluster | GW 55 | $23.7\pm5.2^{cd**}$ | 0.02 | 0.88 | | | | |

### 3.3.2 Sensory perception of non-clustered and hetero-aggregated clustered o/w emulsions

Table 3 shows the mean intensity scores of RATA ratings for mouth-feel (3a) and after-feel attributes (3b). Both cluster size and cluster strength significantly influenced thickness intensity. Emulsions with large clusters (GW55, GD55) were perceived significantly thicker than non-clustered emulsions (GD01, GD10, GW01, GW10). Weakly bound clusters (GW55) were perceived significantly less thick then strongly bound clusters (GD55). The increase in thickness is in agreement with the increase in viscosity of o/w emulsions upon clustering. Similar to thickness, creaminess intensity was significantly higher for clustered emulsions than for non-clustered emulsions (GD01, GD10, GW10, and GW01). Creaminess intensity of clustered emulsions ranged between 3.7 and 5.4, whereas non-clustered emulsions had intensities of around 3.0. For strongly bound clusters (GD19, GD37, GD55), creaminess intensities of 3.8, 4.0 and 4.4 were found and thus increased with cluster size. For weakly bound clusters (GW19, GW37 and GW55), creaminess intensities of 3.7, 4.7 and 5.4 were observed. Non-clustered emulsions thickened with xanthan (GD01_Xan, GW01_Xan) had comparable thickness and creaminess intensities as emulsions with large clusters (GD55, GW55). Weakly bound clusters (GW) were perceived as significantly creamier than strongly bound clusters (GD). This demonstrates that creaminess cannot be explained entirely by thickness and viscosity of o/w emulsions, but also cluster strength and cluster structure need to be taken into account. Besides creaminess and thickness, cluster strength also affected graininess perception. For strongly bound clustered o/w emulsions (GD), graininess significantly increased with increasing cluster size. Clusters (GD) ranging from 5 to 40 μm (GD19, GD37, GD55) increased in graininess intensity from 2.8 for small clusters (GD19) to 5.0 for large clusters (GD55). This increase in graininess intensity was not observed for weakly bound clustered o/w emulsions (GW). For all weakly bound clustered emulsions (GW19, GW37, GW55), graininess intensity was not significantly different from that for non-clustered o/w emulsions (GW01, GW10). This suggests that cluster size seems to be less critical for graininess perception. However, the cluster interaction strength of oil droplets seems to be the main driver for graininess perception in these emulsions. Figure 5 shows the PCA of the sensory perception of all o/w emulsions. The first two principal components explain more than 73% of the variation in the data and two main descriptive axes were found. The horizontal axis separates emulsions mainly from watery to thick. The vertical axis separates emulsions mainly from grainy and astringent to sweet and vanilla.
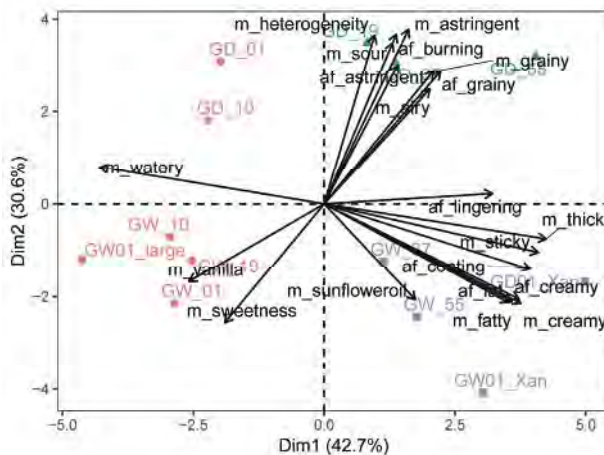
**Figure 5. PCA bi-plot of RATA ratings (n=83 subjects) of the studied emulsions. Groups of o/w emulsions are indicated by different colours (red, green and blue). The central point of each group is displayed as a large symbol (red dot, green triangle, blue square).**

A hierarchical cluster analysis shows the presence of three cluster families, which are highlighted in Figure 5 by different colours (red, green and blue). These cluster families are well separated in the PCA. On the left side, the homogenous non-clustered o/w emulsions are grouped (red). These emulsions are mainly described by a watery mouth-feel. Emulsions with non-clustered droplets, but increased droplet size (GW01_large, 41 μm) are also located in this cluster. Emulsions with large, weakly bound clusters (GW37, GW55) are part of a separate cluster (blue) and are mainly related to thick, coating and creamy descriptors. With increasing cluster size, so moving from GW01 and GW10 towards GW55, both thick and creamy mouth-feel increase significantly. This shows that oil droplet clustering leads to an enhancement of these fat-related attributes. In the same group (blue), emulsions thickened with xanthan (GW01_Xan, GD01_Xan) are present.

Clearly separated from those emulsions are the gelatine-DATEM (GD) emulsions with strongly bound clusters, which form the third group in the PCA (green) in the upper right quadrant. While also for these emulsions thickness increased and wateriness decreased, they were mainly perceived as grainy, astringent and sour. This is most likely related to the structure of the clusters, as well as the properties of the emulsifier (inherent sour taste of DATEM). While both GW and GD clusters had a comparable size, the interaction strength among oil droplets within the cluster differed. This might have led to different behaviour in the mouth during oral processing eliciting different sensations. For example, for weakly bound GW clustered emulsions, oil droplet clusters might fall apart due to interactions with saliva, or because of the applied shear during consumption. This may lead to a decrease in viscosity during consumption. However, even though viscosity decreased, creaminess did not. Clusters that may stay intact in the mouth, like for example those present in GD55 (strongly bound), may be so large and stiff that they might be sensed as a particle and consequently, the emulsion was perceived as grainy. Also, the interaction with saliva and the negatively charged mucins therein can contribute to graininess perception. The various

emulsifiers interact differently with saliva. Gelatine is positively charged in these emulsions, thus in combination with negatively charged salivary mucins, electrostatic attraction between gelatine-covered droplets and mucins occurs (see section 3.3.3). Thus, clusters may fall apart upon the addition of saliva, due to competition with negatively charged, DATEM-stabilised droplets.

However, saliva could also assist in the aggregation of non-clustered droplets. DATEM and whey protein, on the other hand, are negatively charged in the emulsions, and the repulsion between the clusters and the mucins may result in depletion interactions. Both flow and friction behaviour can be strongly changed by the interaction of the emulsions with saliva during consumption and might help to understand the perception of the clustered systems. To conclude, oil droplet cluster size and cluster strength are the two main physical parameters that separate the hetero-aggregated clustered o/w emulsions over the sensory space. Clustering of oil droplets in o/w emulsion alters not only the rheological and tribological properties but also the sensory perception of o/w emulsion and can be used to enhance the perception of fat-related sensory attributes. This finding suggests that the incorporation of the aqueous phase inside the oil droplet cluster leading to an increased oil volume fraction affects sensory perception comparable to the incorporation of water droplets into w/o/w emulsions. For hetero-aggregated o/w emulsions with oil droplet clusters as well as for w/o/w emulsions, the increased volume fraction of the dispersed phase has a positive influence on sensory perception of fat-related attributes (Oppermann, Renssen, Schuch, Stieger, & Scholten, 2015; Oppermann, Piqueras-Fiszman, de Graaf, Scholten, & Stieger, 2016; Oppermann, Verkaaik, Stieger, & Scholten, 2017).

**3**

**Table 3a. Mean RATA ratings of (a) mouth-feel attributes (n=83 subjects) and (b) after-feel attributes with standard error (SE) for the studied emulsions. Means with the same superscript letter (Tukey-HSD test) are not significantly different at p<0.05. F and p-values indicate the variation within one attribute (for all attributes p<0.001).**

| mouthfeel attributes | airy | astringent | creamy | fatty | grainy | heterogeneity | sour | sticky | sunflower oil | sweetness | thick | vanilla | water |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F=12.5 | F=10.0 | F=47.8 | F=21.5 | F=45.8 | F=3.3 | F=60.8 | F=20.7 | F=4.7 | F=8.2 | F=77.6 | F=6.8 | F=51.1 |
| GD01_Xan | 2.6±0.2 d | 2.7±0.3 abc | 5.7±0.2 a | 5.4±0.2 a | 1.2±0.2 d | 3.1±0.3 bc | 4.7±0.3 ab | 3.5±0.3 a | 3.4±0.3 a | 2.1±0.2 b | 5.7±0.2 ab | 2.0±0.2 a | 2.8±0.2 e |
| GD_01 | 2.9±0.3 cd | 2.9±0.3 ab | 2.7±0.2 fg | 3.0±0.2 f | 1.2±0.2 d | 3.3±0.3 abc | 5.4±0.3 a | 1.6±0.2 f | 2.6±0.3 bc | 2.0±0.2 b | 2.2±0.2 f | 2.1±0.2 a | 6.0±0.2 a |
| GD_10 | 2.4±0.2 d | 3.0±0.3 ab | 3.2±0.2 efg | 3.3±0.2 ef | 1.0±0.1 d | 3.2±0.3 abc | 4.9±0.3 ab | 1.6±0.2 f | 3.0±0.2 abc | 2.1±0.2 b | 2.4±0.2 f | 2.5±0.2 a | 5.9±0.2 a |
| GD_19 | 3.6±0.3 bc | 3.0±0.3 ab | 3.8±0.2 cde | 3.9±0.2 cdef | 2.8±0.3 c | 4.3±0.3 a | 4.8±0.3 ab | 2.2±0.2 cdef | 2.8±0.3 abc | 2.0±0.2 b | 3.4±0.2 de | 2.0±0.2 a | 4.8±0.2 bc |
| GD_37 | 4.2±0.3 b | 3.0±0.3 a | 4.0±0.2 cde | 4.1±0.3 bcde | 3.8±0.4 b | 3.8±0.3 abc | 4.1±0.3 b | 2.4±0.3 cde | 2.9±0.2 abc | 2.2±0.2 ab | 4.1±0.3 cd | 2.0±0.2 a | 4.4±0.3 cd |
| GD_55 | 5.1±0.3 a | 3.0±0.2 ab | 4.4±0.3 cd | 4.8±0.2 ab | 5.0±0.3 a | 4.2±0.3 ab | 4.1±0.3 b | 3.1±0.3 ab | 2.9±0.2 ab | 2.2±0.2 ab | 5.8±0.3 a | 2.1±0.2 a | 3.0±0.2 e |
| GW_01 | 2.9±0.3 cd | 1.8±0.2 d | 3.7±0.2 de | 3.8±0.2 def | 1.0±0.1 d | 2.9±0.3 c | 1.8±0.2 c | 1.9±0.2 ef | 3.0±0.2 ab | 2.5±0.2 a | 2.4±0.2 f | 2.5±0.2 a | 5.9±0.3 a |
| GW_10 | 2.8±0.3 cd | 1.8±0.2 d | 3.6±0.2 def | 3.7±0.2 def | 1.0±0.2 d | 3.4±0.3 abc | 1.7±0.2 c | 1.7±0.2 ef | 2.2±0.2 c | 2.5±0.2 ab | 3.0±0.2 ef | 2.2±0.2 a | 5.5±0.3 ab |
| GW_19 | 2.7±0.3 cd | 2.2±0.3 bcd | 3.7±0.2 cdef | 3.9±0.2 cdef | 1.0±0.2 d | 3.2±0.3 abc | 1.9±0.2 c | 1.7±0.2 ef | 3.0±0.2 abc | 2.6±0.2 ab | 2.7±0.2 ef | 2.1±0.2 a | 5.8±0.3 ab |
| GW_37 | 3.3±0.2 bcd | 2.1±0.2 cd | 4.7±0.2 bc | 4.3±0.2 bcd | 1.3±0.2 d | 3.2±0.3 bc | 1.9±0.2 c | 2.7±0.3 bcd | 3.0±0.3 ab | 2.4±0.2 ab | 4.2±0.2 cd | 2.3±0.2 de | 4.1±0.2 cd |
| GW_55 | 3.3±0.2 bcd | 2.1±0.2 cd | 5.4±0.2 g | 4.7±0.2 abc | 1.3±0.2 d | 3.2±0.3 bc | 1.8±0.2 c | 2.8±0.2 abc | 2.9±0.2 abc | 2.5±0.2 ab | 4.9±0.2 bc | 2.3±0.2 de | 3.7±0.2 de |
| GW01_large | 2.8±0.3 cd | 1.9±0.2 d | 2.6±0.2 g | 4.1±0.2 bcde | 1.4±0.2 d | 3.3±0.3 abc | 1.4±0.2 c | 2.0±0.2 def | 3.2±0.2 ab | 2.4±0.2 ab | 2.2±0.2 f | 2.3±0.2 a | 6.2±0.3 a |
| GW01_Xan | 2.6±0.2 d | 1.9±0.2 cd | 5.5±0.2 ab | 5.3±0.2 a | 1.3±0.2 d | 2.9±0.3 c | 1.6±0.2 c | 2.9±0.2 abc | 3.4±0.3 a | 2.2±0.2 ab | 5.2±0.2 ab | 2.2±0.2 a | 3.1±0.2 e |

**3**

Table 3b. Mean RATA ratings of after-feel attributes with standard error (SE) for the studied emulsions. Means with the same superscript letter (Tukey-HSD test) are not significantly different at p<0.05. F and p-values indicate the variation within one attribute (for all attributes p<0.001).

| after-feel attributes | astringent | burning | coating | creamy | fatty | grainy | lingering |
|---|---|---|---|---|---|---|---|
| | F=6.8 | F=7.1 | F=9.6 | F=38.7 | F=16.5 | F=19.6 | F=6.1 |
| GD01_Xan | 3.0±0.3 [abc] | 1.9±0.3 [abcde] | 4.8±0.2 [a] | 5.5±0.2 [a] | 5.6±0.2 [a] | 1.2±0.2 [d] | 4.8±0.3 [a] |
| GD_01 | 3.3±0.3 [ab] | 2.4±0.3 [a] | 3.5±0.2 [cd] | 3.6±0.2 [ef] | 4.1±0.3 [cd] | 1.3±0.2 [d] | 4.2±0.3 [ab] |
| GD_10 | 3.4±0.3 [a] | 2.1±0.3 [abcd] | 3.7±0.2 [bcd] | 3.4±0.3 [ef] | 3.7±0.2 [cd] | 1.2±0.2 [d] | 4.4±0.3 [ab] |
| GD_19 | 3.1±0.2 [abc] | 2.2±0.3 [ab] | 3.7±0.2 [cd] | 3.8±0.2 [de] | 4.4±0.2 [bcd] | 2.2±0.3 [bc] | 4.1±0.2 [ab] |
| GD_37 | 3.0±0.3 [abc] | 2.1±0.2 [abc] | 3.9±0.3 [bc] | 4.0±0.3 [cde] | 4.0±0.3 [cd] | 2.7±0.3 [b] | 4.1±0.3 [ab] |
| GD_55 | 3.1±0.3 [abc] | 2.1±0.3 [abc] | 4.2±0.3 [abc] | 4.4±0.3 [bcd] | 4.4±0.3 [bcd] | 3.6±0.3 [a] | 4.2±0.3 [ab] |
| GW_01 | 2.7±0.3 [abcd] | 1.4±0.2 [bcdef] | 3.7±0.2 [bcd] | 4.0±0.3 [cde] | 4.2±0.3 [bcd] | 1.2±0.2 [d] | 3.8±0.3 [b] |
| GW_10 | 2.5±0.2 [bcd] | 1.3±0.2 [cdef] | 3.6±0.3 [cd] | 3.8±0.2 [de] | 3.6±0.3 [d] | 1.2±0.2 [d] | 4.0±0.3 [ab] |
| GW_19 | 2.6±0.3 [abcd] | 1.5±0.2 [bcdef] | 3.7±0.2 [bcd] | 3.9±0.2 [cde] | 4.2±0.3 [bcd] | 1.3±0.2 [d] | 3.7±0.3 [bc] |
| GW_37 | 2.8±0.2 [abc] | 1.9±0.2 [abcdef] | 4.3±0.2 [abc] | 4.8±0.3 [abc] | 4.5±0.3 [bc] | 1.3±0.2 [d] | 4.2±0.2 [ab] |
| GW_55 | 2.8±0.2 [abc] | 1.3±0.2 [def] | 4.3±0.2 [abc] | 5.0±0.2 [ab] | 5.1±0.3 [ab] | 1.5±0.2 [cd] | 4.2±0.3 [ab] |
| GW01_large | 1.9±0.2 [d] | 1.1±0.2 [f] | 2.9±0.2 [d] | 2.6±0.2 [f] | 3.5±0.3 [d] | 1.1±0.2 [d] | 2.9±0.2 [c] |
| GW01_Xan | 2.3±0.2 [cd] | 1.24±0.2 [ef] | 4.6±0.3 [ab] | 5.5±0.2 [a] | 5.6±0.2 [a] | 1.3±0.2 [d] | 4.6±0.3 [ab] |

### 3.3.3 Effect of saliva addition on rheological and tribological properties of non-clustered and clustered o/w emulsions

Table 2 (b) provides an overview of oil droplet and cluster size, rheological and tribological properties of the non-clustered and clustered o/w emulsions mixed with human saliva in a 1:1 ratio. It can be argued that this mixing ratio might lead to an overestimation of the *in vivo* effect of saliva on the emulsions. However, the described experiments were meant as an investigation on the effect of saliva on the properties of the emulsions. Under realistic drinking conditions, the oral residence time of liquid emulsions is likely to be <5s per sip, and the emulsion:saliva mixing ratio is probably shifted towards more emulsion and less saliva. For the gelatine-stabilised emulsions (GD10, GW10), the droplet size increased from 1-2 μm to > 25 μm upon mixing with saliva. This increase in droplet size was most likely due to flocculation of positively charged gelatine-stabilised droplets with negatively charged salivary proteins (mucins). Negatively charged emulsions emulsified with DATEM or whey protein (GD01, GW01) showed no changes in droplet size upon mixing with saliva.

Because flocculation occurred upon mixing with saliva for emulsions with positively-charged droplets (GD10, GW10), the viscosity of these emulsions increased. For GD10, consistency K increased from 0.01 to 0.17 Pa·s$^n$; for GW10, K increased from 0.005 to 0.25 Pa·s$^n$. For both emulsions, the flow index n decreased from 0.88 (GD10) and 0.96 (GW10) to roughly 0.40 upon mixing with saliva, indicating the emulsions became strongly shear thinning. Consistency K of negatively charged non-clustered emulsions stabilised with whey (GW01) increased from 0.002 to 0.01 Pa·s$^n$, which is consistent with the limited change in droplet size. However, for the DATEM-stabilised non-clustered emulsion GD01, K increased largely from 0.002 to 0.18 Pa·s$^n$, even though droplet size did not change. The increase in viscosity might be caused by depletion or repulsive interactions hindering flow at low shear rates as DATEM-stabilised droplets have a higher charge density than WPI-stabilised droplets.

Emulsions GD55 and GW55 with large clusters showed a reduction in effective cluster size upon addition of saliva from around 40 μm to 25-36 μm. Cluster size decreased slightly, as the negatively charged proteins present in saliva likely interacted with gelatine covered droplets, inhibiting binding among oil droplets and thereby (re-)formation of clusters. Although effective cluster size was reduced only slightly, the viscosity of the emulsions decreased considerably. K of GD55 decreased from 2.80 to 0.09 Pa·s$^n$, while GW55 decreased from 1 to 0.02 Pa·s$^n$. This decrease in viscosity was accompanied by a decrease in cluster strength. Emulsions with large gelatine-DATEM clusters showed a reduction of critical strain from about 4.1 to 0.25% upon addition of saliva, and a similar trend was seen in gelatine-WPI emulsions. We suggest that these changes in viscosity and cluster strength are indicative of both the dilution of the emulsions with saliva and changes in the cluster properties (*e.g.* cluster structure and competition for electrostatic interactions between salivary proteins and droplets). Emulsions thickened with xanthan (GD01_Xan, GW01_Xan) did not show changes in droplet size upon mixing with saliva. This is linked to the negative charge of the droplets, which does not allow electrostatic attraction and subsequent droplet flocculation. For these emulsions, K decreased from 1.9 to 0.25 Pa·s$^n$ for GD01_Xan and from 2 to 0.26 Pa·s$^n$ for GW01_Xan. Emulsions diluted with water in a 1:1 ratio showed a comparable reduction in K to about 0.25 Pa·s$^n$, suggesting that the reduction can be attributed to dilution.

Also, the friction behaviour was affected by the addition of saliva. The friction coefficient of positively charged, gelatine-stabilised emulsions (GD10, GW10) decreased slightly in the boundary regime

($\mu_{bound}$) but did not change in the intermediate and high-speed regime ($\mu_{10mm/s}$, $\mu_{80mm/s}$). This is in contrast with the changes in droplet size. Upon addition of saliva, droplet size increased strongly due to flocculation. Clustering was previously seen to decrease friction. Apparently, saliva can increase friction. Also, in the case of negatively charged non-clustered emulsions (GD01, GW01), saliva seemed to increase friction. For these emulsions, an overall increase in friction coefficient was observed upon addition of saliva (Table 2b), whereas the cluster size remained constant. Laguna and co-workers (Laguna et al., 2017) reported a comparable observation of the effect of saliva on friction. They showed that the addition of artificial saliva to skimmed milk increased the friction coefficient. This increase in friction was ascribed to the adherence of the saliva proteins to surfaces of the tribo-pair. Dresselhuis et al. (2007) suggested that proteins in saliva might act as additional components in the bulk and adhere to either surface of the tribo-pair, and consequently increase friction. This is in line with our findings. Negatively charged salivary proteins are not expected to interact with negatively charged emulsion droplets but may adhere to the surfaces of the tribo-pair and increase friction.

In the case of clustered emulsions, the friction coefficients showed an overall increase with the addition of saliva. This may be related to changes in the cluster properties. However, a conclusive explanation of the interplay among emulsion properties, saliva, and friction cannot be provided. Further research on the relation between saliva, aggregation and lubrication behaviour of emulsions needs to be performed.

To conclude, the addition of saliva changes droplet size, rheological and tribological properties of both non-clustered and clustered emulsions. Positively charged non-clustered emulsions tend to flocculate and thereby viscosity increases, whereas negatively charged non-clustered emulsions tend to be less affected by saliva addition. For clustered emulsions, mixing with saliva reduces viscosity, which is mainly related to a dilution effect. Friction coefficients generally increase for clustered emulsions.

### 3.3.4 Correlations between sensory perception and physical characteristics of hetero-aggregated clustered o/w emulsions

To investigate how sensory attributes are related to emulsion characteristics, correlations between physical parameters describing physical changes upon saliva addition and RATA scores were determined (Table 4). Only mouthfeel attributes related to fat perception during oral processing are presented. We see similar trends for after-feel, but we focus the discussion on mouthfeel attributes. Physical parameters that were not correlated to sensory attributes are not dealt with. First, correlations of sensory data with physical descriptors of the o/w emulsions are discussed and, subsequently, correlations of the sensory data with physical descriptors of emulsions containing saliva (saliva addition is indicated in the table with a "(+)"). As expected, thickness correlated strongly with consistency K. This is in line with several previous studies showing that perceived thickness is highly viscosity dependent (Akhtar et al., 2005; Camacho, Dop, de Graaf, & Stieger, 2015; Christensen & Casper, 1987; van Aken et al., 2011). Most fat-related attributes were strongly correlated to both rheological (viscosity, flow index and consistency) and tribological parameters. When friction decreased ($\mu_{80mm/s}$ and $\mu_{10mm/s}$), creaminess and fattiness increased which is in agreement with literature (Chojnicka-Paszun, de Jongh, & de Kruif, 2012; de Wijk et al., 2011; de Wijk & Prinz, 2005; van Aken et al., 2011).

When taking into account the changes in the physical characteristics of the emulsions upon addition of saliva, some of the correlations between sensory attributes and rheological properties of emulsions

disappeared. Consistency K and thickness did not correlate anymore when emulsions were mixed with saliva. Instead, as a result of saliva addition, the tribological parameter describing the slope of the mixed regime (b exponent) correlated with specific sensory attributes (graininess, fattiness and stickiness), suggested to be associated with later stages of oral processing (de Wijk et al., 2011). The exponent b represents changes in friction coefficient of emulsions with increasing speed in the mixed regime. The lower the exponent b, the larger the changes in the emulsions, which might be related to a higher affinity to form a film and faster formation of an oil film. Therefore, the emulsions might be perceived as more fatty. If emulsions can form a film more easily by droplet coalescence, as is especially the case for DATEM-stabilised emulsions, lubrication is improved.

In conclusion, rheological properties correlate strongly with thickness and creaminess, which are considerably enhanced by clustering. Friction properties correlate with fattiness, graininess and stickiness, especially when saliva addition is taken into account. The creaminess was found to correlate to tribological properties in the absence of saliva. This provides clear indications that oral processing has to be taken into account when relating physical measurements to sensory perception, especially in different stages of consumption.

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**Table 4. Overview of correlations between sensory attributes (mouthfeel) (white) and physical parameters of o/w emulsions (light grey). Category names containing (+) (dark grey) represent o/w emulsions mixed with saliva. Correlation coefficient and p-values are shown (p<0.05 "*", p<0.01 "**", p<0.001 "***"). Boxes without numbers were not significantly (p>0.05)**

| | airy | sour | sweet | astringent | thick | grainy | creamy | fatty | watery | sticky | effect. cluster size | viscosity (at 50/s) | consistency K | flow index n | $\mu_{20}$ mm/s | $\mu_{40}$ mm/s | b-exponent | effect. cluster size (+) | consistency K (+) | flow index n (+) | viscosity (at 50/s) (+) | $\mu_{20}$ mm/s (+) | $\mu_{40}$ mm/s (+) | b-exponent (+) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| b-exponent (+) | | | | | | | | | | | | | | | | | | | | | | -0.62 * | | |
| $\mu_{40}$ mm/s (+) | 0.80 ** | | | | | | | | | | | | | | | | | | | | | 0.84 * | | |
| $\mu_{20}$ mm/s (+) | 0.69 * | | | | | | | | | | | | | | | | | | | | | | | |
| viscosity (at 50/s) (+) | | | | | | | | | | | | | | | | | | | 0.84 ** | -0.62 * | | | | |
| flow index n (+) | | | | | | | | | | | | | | | | | | | -0.78 ** | | | | | |
| consistency K (+) | | | | | | | | | | | | | | | | | | | | | | | | |
| effect. cluster size (+) | 0.68 * | | | | | | | | | | | | | | | | | | | | | | | |
| b-exponent | | | | | | -0.74 * | -0.70 * | -0.61 * | | -0.64 ** | | | | | | | | | | | | | | |
| $\mu_{40}$ mm/s | | | | | -0.78 ** | | -0.67 * | -0.68 * | 0.79 ** | -0.65 * | -0.69 * | -0.63 * | -0.65 * | 0.76 ** | | | | | | | | | | |
| $\mu_{20}$ mm/s | | | | | | | -0.68 * | -0.61 ** | | -0.66 * | | -0.61 * | | 0.61 * | | | | | | | -0.61 * | | | |
| flow index n | | | | -0.61 * | -0.77 ** | -0.62 * | | -0.77 ** | 0.76 ** | -0.79 ** | | -0.8 ** | -0.73 ** | 0.64 * | 0.65 * | | | | | | | | | |
| consistency K | | | | | 0.91 *** | | 0.77 ** | 0.85 ** | -0.92 *** | 0.87 * | 0.77 ** | -0.82 ** | -0.9 ** | | | | | | | | | | | |
| viscosity (at 50/s) | | | | | 0.91 *** | | 0.83 ** | 0.84 ** | -0.95 ** | 0.85 * | | 0.97 *** | | | | | | | | | | | | |
| effect. cluster size | | | | | | 0.72 ** | 0.85 ** | 0.99 *** | -0.88 ** | 0.85 * | -0.68 * | | | | | | | | | | | | | |
| sticky | | | | | 0.91 ** | | -0.92 ** | -0.87 ** | | | | | | | | | | | | | | | | |
| watery | | | | | -0.98 *** | | 0.88 ** | | | | | | | | | | | | | | | | | |
| fatty | | | | | 0.89 ** | | | | | | | | | | | | | | | | | | | |
| creamy | 0.61 * | | | | 0.9 ** | | | | | | | | | | | | | | | | | | | |
| grainy | | | | | | | | | | | | | | | | | | | | | | | | |
| thick | | | | | | | | | | | | | | | | | | | | | | | | |
| astringent | | 0.75 ** | -0.76 ** | | | | | | | | | | | | | | | | | | | | | |
| sweet | | -0.76 ** | | | | | | | | | | | | | | | | | | | | | | |
| sour | | | | | | | | | | | | | | | | | | | | | | | | |
| airy | | | | | | | | | | | | | | | | | | | | | | | | |

## 3.4. Conclusions

Hetero-aggregation of oil droplets strongly influences physical, rheological and tribological properties of o/w emulsions. Due to an increase in effective volume fraction, viscosity increases strongly by oil droplet clustering. Due to an increase in viscosity, clustered o/w emulsions are perceived as thicker and creamier compared to o/w emulsions with non-clustered oil droplets. These attributes are enhanced with increasing cluster size. The strength of the clusters affects sensory properties. Emulsions with weakly bound clusters (gelatine-whey protein) are perceived as creamy, whereas emulsions with strongly bound clusters (gelatine-DATEM) are perceived as grainy, even though cluster size is similar. Strongly bound clusters show a steeper decrease of the friction coefficient in the mixed regime. Lubrication behaviour of hetero-aggregated clustered o/w emulsions thus depends on cluster size and strength. Upon clustering, emulsions become better lubricants than non-clustered emulsions. Without saliva addition, thickness and creaminess perception correlate well with rheological and tribological properties of o/w emulsions. In contrast, with saliva addition, graininess and fattiness perception are stronger related to tribological properties suggesting that oral processing and the accompanying changes in emulsion structure as a result of mixing with saliva are important contributors to sensory perception. We conclude that clustering of oil droplets in o/w emulsions can be used as a tool to control the sensory perception of emulsions and enhance the perception of fat-related attributes.

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## Appendix

Figure A.1 shows examples of Stribeck curves of non-clustered emulsions (GD01, GD10, GW01, GW10, and GD01_Xan) and clustered emulsions (GD55 and GW55).
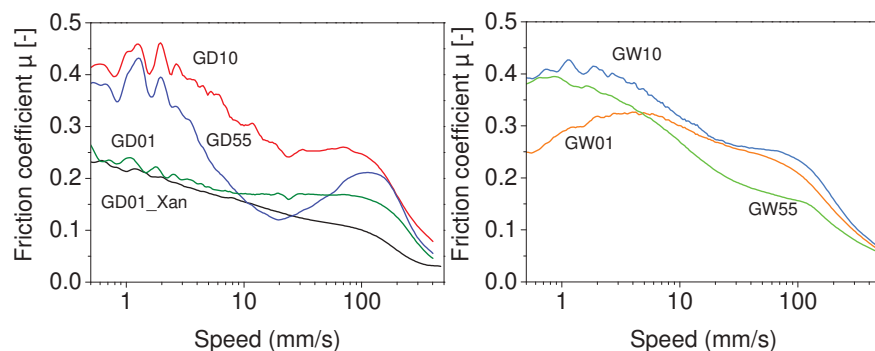


**Figure A.1 Friction coefficient as a function of speed for non-clustered and clustered o/w emulsions for gelatine-DATEM and gelatine-WPI clustered emulsions. (left figure) The red top line shows a homogeneous gelatine stabilised emulsion (pH 5, GD10), the green line shows a homogeneous DATEM stabilised emulsion (GD01), the blue line shows a highly clustered gelatine-DATEM emulsion (GD55), the black line shows a homogeneous DATEM stabilised emulsion containing 0.3% Xanthan (GD01_Xan). (right figure) The blue line shows a homogeneous gelatine stabilised emulsion (pH7, GW10), the green line shows a highly clustered gelatine-WPI emulsion (GW55), and the red line shows whey protein isolate stabilised emulsion (GW01). Curves are obtained from averaging 3 measurements.**

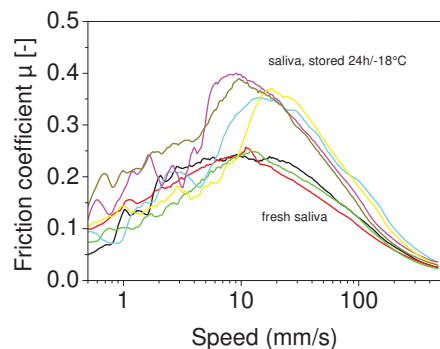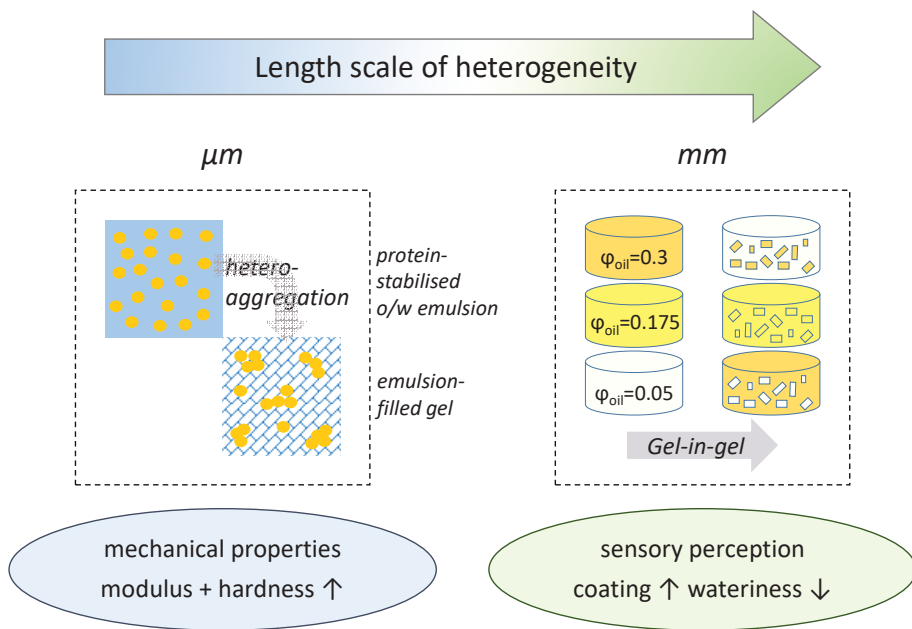Figure A.2 shows exemplary Stribeck curves of fresh saliva and saliva stored for 24 hours at -18 °C.



**Figure A.2 Friction coefficient as a function of speed for fresh saliva and saliva stored for 24 hours at -18 °C. Curves are obtained from averaging 3 measurements.**

**Table A3: Overview of sensory terms and definition used for RATA evaluation of all emulsions by n=83 subjects. Definitions were taken from Oppermann et al. (2017); van Aken et al. (2011) and Benjamins et al. (2009) and adjusted where required.**

| Sensory attributes with descriptions | |
|---|---|
| *Mouth-feel* | *After-feel* |
| **Thick** | **Creamy** |
| Intensity of thickness of the product in the mouth after taking a bite/sip. This attribute is perceived by moving the tongue up and down against the palate. This moving up and down is especially important for more thick products. If a product is very thin, it immediately spreads throughout the mouth. From water to yoghurt thickness | Degree to which a product leaves a soft creamy after-feel after swallowing. It is related to the creamy/soft mouthfeel sensation but is less intense. |
| **Airy** | **Fatty** |
| Degree to which a product is airy. The product feels light on the tongue and is a bit foamy. It takes little effort to take it apart. This taking apart is done with the tongue. Whipped egg white is very airy (maximum score) and is not coherent; it takes little effort to take it apart. Airy is perceived between the tongue and the palate. | The degree to which a product leaves a fatty feeling in the mouth. This is a typical coating in the mouth. It can be felt on the teeth and palate by moving the tongue alongside them. |
| **Grainy** | **Burning** |
| Degree to which a product contains grains. This attribute can be judged by rubbing the tongue against the palate. Air bubbles as granules as semolina pudding | Intensity of the burning feeling after swallowing a spicy or sour product. Burning, prickling after-feel at the back of the throat |
| **Creamy** | **Astringent** |
| The intensity of the creaminess/softness. This is a soft, full feeling in the mouth, which is also thick-soft and supple. The product is not rough feels not dry. It leaves a soft, fatty feeling and is often in combination with fat. It is perceived in the whole mouth and gives a velvety feeling in the whole mouth. Flows through the mouth; velvety; warm; soft | The intensity of astringency after swallowing or expectorating the product. It is the tart or rough feeling in the mouth that remains after eating, for example, spinach, whine, or rhubarb. The feeling is perceived in the whole mouth. Contracting after-feel |
| **Sticky** | **Coating** |
| Intensity of stickiness. This is a sticky feeling that can be perceived with tongue and palate. An example of a really sticky product is the caramel of a Mars candy bar. It is perceived between the teeth during a chew movement. The degree to which a product comes loose from different parts of the mouth determines the intensity of stickiness. Sticky but you get strands in the mouth | The degree to which a product leaves a feeling in the whole mouth. It can be felt on the teeth and the palate. |
| **Heterogeneity** | **Grainy** |
| Intensity of the feeling of heterogeneity. This is the feeling you get from a product that is both thin and thick at the same time. The feeling can also be cloudy or flake-like. It occurs if a product does not melt evenly after putting it into the mouth. Sometimes it takes a while before it can be perceived. The feeling is perceived in the whole mouth. | Feeling like particles remain in mouth, can be smaller or bigger. Like small salt particles, bubbles, semolina. |
| **Fatty** | **Lingering** |
| Intensity of fatty feeling in the mouth. It gives a smooth feeling and a coating on the palate. At first, it is perceived on the inside of the edge between teeth and gums and later it is also perceived on the outside. Fatty layer that stays in the mouth | A feeling that sample or the perception of the sample stays long in mouth |
| **Watery** | |
| Consisting of containing water, Thin, weak texture, opposite of viscous, lacking body | |
| **Astringent** | |
| Intensity of astringent or rough feeling in the mouth. A chalky, rough feeling in the mouth (especially on the teeth) like eating nuts or spinach. It can be perceived in the following places: at the front of the palate (border gum and teeth), at the back of the throat, at the back of the tongue, and at the cheeks. | |
| **Sour** | |
| Intensity of sour flavour | |
| **Vanilla** | |
| Intensity of vanilla flavour. taste of vanilla | |
| **Oil flavour** | |
| Intensity of oily, fatty flavour. Taste of salad oil or fat | |
| **Sweet** | |
| Intensity of sweet flavour. Sweet taste | |

# Chapter 4 - Effect of oil droplet inhomogeneity at different length scales on mechanical and sensory properties of emulsion-filled gels: Length scale matters

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## Abstract

We studied the effect of inhomogeneity in oil droplet distribution at different length scales on the
mechanical and sensory properties of emulsion-filled food gels. Two approaches were followed to obtain
inhomogeneous distributions at different length scales: (1) clustering of o/w-emulsions by hetero-
aggregation and subsequent gelation to obtain inhomogeneity at µm-scale, and (2) incorporating particles
of emulsion-filled gels into emulsion-filled gel matrices with a different volume fraction of oil droplets to
obtain gel-in-gels with inhomogeneity at mm-scale.

Upon clustering of oil droplets at µm-scale, the Young's modulus of the gels increased by up to 60%,
whereas fracture stress and strain depended on emulsifier-matrix interactions. Clustering of oil droplets
affected mainly the perception of texture-related sensory attributes, such as hardness, but did not
significantly affect the perception of fat-related sensory attributes. Fat-related sensory attributes, such as
creaminess and melting, were dominated by emulsifier matrix interactions.

For gel-in-gels, the inhomogeneous distribution of oil droplets at mm-scale did not affect Young's modulus
or fracture strain. The incorporation of particles decreased the fracture stress of the gels, independently
of the droplet distribution. The perception of fat-related sensory attributes changed significantly. Oiliness
was lower in samples with lower oil content in the outer phase of the gel than in the inner dispersed
particles. In contrast, coating perception increased in samples in which the oil droplet distribution was
inhomogeneous, independently on whether the outer phase or the inner gel particles contained a higher
oil volume fraction. The creaminess was only slightly affected.

We conclude that oil droplet clustering at µm-scale can be used to modify mechanical properties and
texture-related perception of emulsion-filled gels. In contrast, inhomogeneity at mm-scale allows altering
fat-related sensations. Sensory perception can be controlled by modifying the interactions between
dispersed oil droplets and matrix using different emulsifiers and by incorporating inhomogeneity in the oil
droplet distribution of emulsion-filled gels at different length scales.

## 4.1 Introduction

The role of dispersed fat/oil droplets on the properties of semi-solid foods has been extensively studied (Dickinson, 2012; Sala, van Vliet, Cohen Stuart, van de Velde, & van Aken, 2009; Sala, van de Velde, et al., 2007b). Fat/oil droplets with varying size and at different concentrations can be incorporated into gel matrices. Depending on the interactions between the emulsifier on the surface of the oil droplets and the matrix, oil droplets act as bound or unbound fillers. All these parameters influence the mechanical properties of emulsion-filled gels. When oil droplets interact with the gel matrix ("bound fillers"), an increase in oil concentration or a decrease in oil droplet size has been shown to increase Young's modulus and perceived hardness (Lett, Norton, & Yeomans, 2016; Chojnicka, Sala, de Kruif, & van de Velde, 2009; Sala, van Aken, Stuart, & van de Velde, 2007b).

Additionally, the spatial distribution of oil droplets within an emulsion-filled gel influences the mechanical properties. Oliver et al. (2016, 2015a, b) changed the spatial distribution of oil droplets by varying the gelation kinetics of the continuous gel phase. When oil droplets were clustered, the Young's modulus of emulsion-filled gels was 1.5x to 2x larger compared to the Young's modulus of emulsion-filled gels with non-clustered oil droplets (Oliver, Wieck, & Scholten, 2016; Oliver, Berndsen, van Aken, & Scholten, 2015b). This effect was mainly attributed to an increase in the effective volume fraction of the dispersed oil phase. The spatial distribution of oil droplets affects not only the mechanical properties of an emulsion-filled gel but also sensory perception. Mosca et al. (2012) demonstrated that the spatial distribution of oil droplets in emulsion-filled gels affect both texture- and fat-related sensory attributes. In the mentioned study, upon combining discrete layers of emulsion-filled gels varying in oil volume fraction (oil droplet inhomogeneity on an mm-scale), perceived firmness decreased, melting increased, and creaminess tended to increase. Thus, inhomogeneity in the distribution of oil droplets at a larger length scale (mm-scale) in emulsion-filled gels alters sensory perception. However, the incorporation of larger layers of fat-rich and fat-depleted layers might not always be feasible; inhomogeneity of oil droplets at smaller length scales could prove useful. Inhomogeneity of oil droplet distribution at small length scales (µm-scale) may thus be used as a strategy to modify texture- or fat-related perception of emulsion-filled gels.

To successfully modify mechanical and sensory properties of fat-containing foods, the control of the extent of the oil droplet inhomogeneity at different length scales is desired. Previously, we described several methodologies to prepare oil droplet clusters with controlled size, strength and morphology in liquid o/w emulsions (Fuhrmann, Sala, Stieger, & Scholten, 2019a). One of these methods is hetero-aggregation, a technique in which electrostatic attraction between oil droplets with oppositely-charged emulsifiers is used to cluster oil droplets in liquid o/w emulsions. Cluster size and cluster strength of hetero-aggregated oil droplets can be controlled using a) (positively-charged) gelatine and (negatively-charged) whey protein or b) (positively-charged) gelatine and (negatively-charged) DATEM as emulsifiers. By changing the ratio between the positively- and negatively-charged emulsion droplets, cluster size can be adjusted, ranging from 5 to 45 µm (Fuhrmann et al., 2019a). Cluster strength is controlled by the strength of the electrostatic attraction between the droplets, which depends on the charge density of the emulsifiers. In liquid o/w emulsions, clustering by hetero-aggregation was shown to enhance the perception of fat-related sensory attributes such as creaminess, thickness and coating perception (Fuhrmann, Kalisvaart, Sala, Scholten, & Stieger, 2019b). Based on these findings, we hypothesise that clustering of oil droplets by hetero-

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aggregation can also be used to enhance the perception of fat-related sensory attributes in semi-solid emulsion-filled gels.

This study aimed to determine the effect of inhomogeneity of oil droplet distribution at different length scales on mechanical and sensory properties of semi-solid gels. To investigate the impact of the length scale of the oil droplet inhomogeneity from µm to mm-scale, gels were prepared following two approaches: (1) clustering of oil droplets by hetero-aggregation of o/w-emulsions and subsequent gelation to obtain emulsion-filled gels with oil droplet clusters at µm-scale, and (2) incorporating particles of emulsion-filled gels into emulsion-filled gel matrices with different oil volume fraction to obtain gel-in-gels with oil droplet inhomogeneity at mm-scale.

## 4.2 Materials and methods

### 4.2.1 Materials
Whey protein isolate (BiPRO, WPI) was bought from Davisco (Lot # JE 062-3-420, USA). Gelatine Type 250 PS 30, with an isoelectric point between pH 8 and 9, was obtained from Rousselot (Lot #1207647, The Netherlands). DATEM and low acyl gellan were kindly provided by CP Kelco (USA). Anhydrous citric acid and sodium hydroxide were obtained from Sigma Aldrich (St. Louis MO, USA). Sunflower oil (Reddy, The Netherlands) was bought from a local retailer. Demineralised water was used (MilliQ® system, Merck Millipore, Germany). Titanium dioxide (TiO2) was purchased from Minerals Water (Rainham, United Kingdom).

### 4.2.2 Oil-in-water emulsions
Non-clustered o/w emulsions

*Whey protein isolate (WPI) stabilised o/w emulsions*
To obtain WPI-stabilised o/w emulsions, 10 mg/mL WPI was dissolved in a citric acid solution (7.5 mM, adjusted to pH 7 with 1 M NaOH) (when the emulsion was used for the preparation of hetero-aggregates) or demineralised water (when the emulsion was used for the preparation of "gel-in-gel" samples) and stirred for 2 hours at room temperature. A stock o/w emulsion with 40% (v/v) oil was prepared by adding sunflower oil slowly to the aqueous phase while pre-homogenizing with a rotor-stator homogeniser (Ultra-Turrax T25, IKA, Germany) at 8000 rpm for 3 minutes. The pre-emulsions were then homogenised at 180 bar for four cycles (LabhoScope, Delta Instruments, The Netherlands). If required, emulsions were subsequently diluted using the corresponding aqueous phase to obtain the desired final oil concentrations. Emulsions were finally stored at 4°C before physical measurements were done.

*Gelatine-stabilised o/w emulsions*
For gelatine-stabilised emulsions (for the preparation of hetero-aggregates), solutions containing 20 mg/mL gelatine in a citric acid solution (7.5 mM, pH 5, adjusted with 1 M NaOH) were prepared, upon heating at 80°C for 30 minutes to dissolve the gelatine. After cooling to 40°C, the pH was adjusted to pH 5 using 1 M NaOH / 1 M HCl, and the oil phase was slowly added while mixing with a rotor-stator homogeniser (Ultra-Turrax T25, IKA, Germany) at 8000 rpm for 3 minutes. The emulsion was further prepared, as explained in the previous section.

*DATEM-stabilised o/w emulsions*

DATEM was dispersed in the oil phase (4 mg/mL oil), and the solution was heated at 80°C for 10 minutes. DATEM solutions were cooled to 40°C. The oil phase was subsequently slowly added to the aqueous phase (citric acid solution pH 5), and the emulsion was prepared as described before. This emulsion was used for the preparation of hetero-aggregated emulsions.

Hetero-aggregated o/w emulsions

Clustered emulsions were obtained by hetero-aggregation, by combining (a) DATEM- and gelatine-stabilised o/w emulsions (pH 5), and (b) WPI- and gelatine-stabilised emulsions (pH 7). After combining the two non-clustered emulsions, samples were stirred, using a magnetic stirrer, and subsequently stored for 24 hours at 4°C before further use. By varying the ratio of positively- and negatively-charged emulsions, clusters of different size were obtained. Combining the clusters at a (volume-)ratio of gelatine-stabilised emulsion to DATEM-stabilised emulsion of 2:8, a cluster size of about 8 μm was obtained. When mixing the emulsions at a ratio 5:5, a size of approximately 50 μm was obtained (also compare Fuhrmann et al., 2019a). The sizes for gelatine-WPI clusters are comparable. In the text, all emulsions are referred to using a 2-letter-2-digit code, where GD refers to emulsions stabilised with gelatine and DATEM, and GW refers to emulsions stabilised with gelatine and WPI. The numbers indicate the mixing ratio between the two oppositely-charged non-clustered emulsions. For example, code GD55 refers to an emulsion consisting of 50% (v/v) gelatine-stabilised emulsion and 50% (v/v) DATEM-stabilised emulsion, while GD01 denotes an emulsion consisting of 100% (v/v) DATEM-stabilised emulsion.

### 4.2.3 Emulsion-filled gels with clustered oil droplets at the μm-scale

Low acyl gellan was used as a gel matrix to prepare gels with hetero-aggregated emulsion droplets. This polysaccharide was chosen based on pre-tests (data not shown), due to its ability to solidify at low temperatures (30°C) and the rather low viscosity of its solutions, making it easy to disperse o/w emulsions in it. Low acyl gellan dispersions were prepared by dispersing 2% (w/v) low acyl gellan in MilliQ water at room temperature. The dispersion was heated at 95°C and stirred for 90 minutes. An aqueous stock solution of 3 M NaCl was added to the gellan solution (at 95°C) to reach a final salt content of 150 mM NaCl. The solution was cooled down to 90°C. The emulsion-filled gel was prepared by combining the gellan solution and the (hetero-aggregated) emulsion at a volume ratio of 1:1. The emulsion and matrix solution were combined under gentle stirring and then poured immediately into a syringe (60 mL volume) placed in an ice bath to allow the gel to set. The obtained gels were stored at 4°C for 12 hours before further processing. Before measurements, gels were equilibrated at room temperature for 2 hours. All emulsion-filled gellan gels containing hetero-aggregated emulsions are coded according to the emulsion used (*e.g.* GD55). The oil concentration was 20% (v/v) in all gels. For calculations, the oil concentration was converted to oil volume fraction (*e.g.* 20% as 0.2, etc.). Samples for sensory evaluation were prepared according to food safety standards in a food-safe environment, using food-grade ingredients. Gels containing hetero-aggregated emulsions were prepared according to the described approach. After preparation, gels were stored at 4°C for a maximum of two days before sensory evaluation. An overview of the samples can be found in Table 1.

**Table 1. Overview of the emulsion-filled gels with hetero-aggregated clusters at µm-scale, including the mixing ratio of the emulsions and approximate cluster size.**

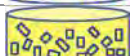| Sample | Sample code | Fraction gelatine-stabilised emulsion | Fraction DATEM-stabilised emulsion | Average droplet/cluster size [µm, in gel] | Oil volume fraction [% (v/v)] | NaCl [mM] |
|---|---|---|---|---|---|---|
| Homogeneous distribution | GD10 | 1 | 0 | 2.5 | | |
| Homogeneous distribution | GD01 | 0 | 1 | 2 | 20 | 150 |
| Small clusters | GD28 | 0.2 | 0.8 | 10 | | |
| Large clusters | GD55 | 0.5 | 0.5 | 20 | | |

4.2.4 Emulsion-filled gels with inhomogeneous oil droplet distribution at mm-scale (gel-in-gels)

Gels with an inhomogeneous droplet distribution at mm-scale were obtained by incorporating particles with a concentration of oil of 5, 17.5 and 30% (v/v), into a continuous gelatine gel matrix with an oil concentration of 5, 17.5 and 30% (v/v). The particles can be regarded as fillers. These particles were prepared by preparing gelatine gels containing WPI-stabilised o/w emulsions. We used gelatine for this approach to ensure efficient incorporation of gel particles into the continuous matrix. First, the gelatine was hydrated in demineralised water for two hours. Subsequently, the suspension was heated to 80°C for 30 minutes to dissolve the gelatine. After cooling the gelatine solution to 40°C, the WPI-stabilised o/w emulsion (preheated to 40°C) was added to the gelatine solution to obtain gels containing an oil concentration of 5, 17.5 and 30% (v/v) in the final system. The gelatine concentration of the aqueous phase was kept constant at 5% (w/v) gelatine. The mixture was stirred and poured into 60 mL syringes and cooled on ice to solidify. After storage at 4°C overnight, samples were further processed. Particles were produced by cutting the gels in round slices of 5 mm height and 26 mm in diameter. These slices were passed through a steel grid with a rectangular mesh (pore size of 1 mm²). The produced particles had an average size of 1x1x5 mm. Particles were stored on ice until used.

Liquid emulsions with gelatine, containing an oil concentration of 5, 17.5 and 30% (v/v) and 5% gelatine in the aqueous phase were prepared. The filler particles, obtained as described previously, were combined in a 1:1 ratio with the liquid emulsion with gelatine in 60 mL syringes. The mixtures were cooled on ice to achieve solidification, and the samples were stored overnight at 4°C. By combining the particles and the liquid emulsion with gelatine, three gels were prepared with a total oil concentration of 17.5%, but different oil distributions. We obtained samples with (a) particles containing 5% (v/v) oil in a continuous phase with 30% (v/v) oil, denoted as 5/30, (b) with particles containing 30% (v/v) oil in a continuous phase with 5% (v/v) oil, denoted as 30/5 and (c) a sample with 17.5% (v/v) oil in both the dispersed particles and the continuous gel, abbreviated as 17.5/17.5. An overview and a schematic drawing of the prepared systems can be found in Table 2.

**Table 2. Overview of the emulsion-filled gelatine gel-in-gels containing mm-scale inhomogeneity of oil droplets.**

| Distribution | Sample code | Oil concentration [%] | | | Mass ratio particle to continuous phase [-] | Schematic depiction |
|---|---|---|---|---|---|---|
| | | Total | Particles | Continuous phase | | |
| Homogeneous | - | 5 | - | 5 | - | |
| | | 17.5 | | 17.5 | | |
| | | 30 | | 30 | | |
| Heterogeneous | 30/5 | 17.5 | 30 | 5 | | |
| Heterogeneous (with equal oil concentration) | 17.5/17.5 | 17.5 | 17.5 | 17.5 | 1:1 | |
| Heterogeneous | 5/30 | 17.5 | 5 | 30 | | |

Samples for sensory evaluation were prepared in a food-safe environment using food-grade ingredients. For gel-in-gels, to avoid visual cues for sensory testing, and to give all samples a similar colour, 0.01% (w/w) $TiO_2$ was added to the aqueous phase of the gels that contained 5% (v/v) oil, and 0.005% (w/w) $TiO_2$ was added to the aqueous phase of the gels that contained 17.5% (v/v) oil, matching the appearance of the gel with 30% (v/v) oil. Further processing of samples was carried out as previously described. After preparation, samples were stored at 4°C for a maximum of two days before sensory evaluation.

### 4.2.5 Structural, physical and mechanical properties of emulsions and emulsion-filled gels

#### Light microscopy and CSLM

Light microscopy pictures (Figure 1A-C) and CLSM pictures (Figure 1D) of the gels were taken by cutting a central specimen of a gel sample. CLSM pictures were taken to confirm that the clusters consisted of individual oil droplets. Samples were prepared as previously described, but the total oil concentration was reduced to 1% to visualise individual clusters more clearly. A slice with a thickness of around 2 mm was cut with a scalpel, using water as a lubricant. Subsequently, the gel was cut into thin slices (~100 μm) on a glass slide; the sample was submerged in demineralised water and covered with a coverslip. Each sample was analysed at six positions using an optical microscope (Axioskop 2 plus, Carl Zeiss AG, Germany) equipped with a camera (Axiocam ERc 5S, Carl Zeiss AG, Germany) and the obtained pictures were analysed with Visio imaging software (Carl Zeiss AG, Germany). For CLSM pictures, the oil phase of emulsion-filled gels was stained using 0.01% (w/v) Nile Red, added to the oil phase before homogenisation. CLSM images were recorded on a LEICA TCS SP5 Confocal Laser Scanning Microscope (Leica Microsystems CMS GmbH, Manheim, Germany) equipped with an inverted microscope (Leica DM IRBE). Samples were excited at 543 nm. The following filters were used: MBS: HFT 488/543 nm (Main Dichroic beam splitter) and DBS2 (secondary beam splitter) at 490 nm. The objective lens used was a Plan-Neofluar 10x/0.3 (Leica). Digital images were acquired at a resolution of 512x512 pixels with an image size of 1270 μm in x and y. Image J/Fiji (1.51s) was used to adapt the contrast and brightness of the microscopic images obtained.

Micrographs were taken of emulsions diluted with their corresponding aqueous phase (without emulsifier). The emulsions were then placed on a microscope slide, and covered with a coverslip. Each sample was analysed for particle size at six positions using an optical light microscope.

### Oil droplet cluster size in emulsions and emulsion-filled gels

The size of oil droplet clusters, in both emulsions and emulsion-filled gels, was determined using microscopy and image analysis. Images were acquired as described above. Recorded images were analysed for particle size using ImageJ (NIH, USA). The scale of a microscopy picture (based on the built-in ZEN imaging software of the Axioskop 2 plus) was used to scale from pixel to μm. The brightness was adjusted automatically, and a threshold of <0.2 μm was applied to remove background noise. An effective cluster diameter was calculated from the cluster area assuming a spherical shape of the clusters. At least three independent sample replicates were used, probed at six positions, and an average effective cluster diameter with standard deviation was calculated and reported.

### Zeta potential

The zeta potential of o/w emulsions was determined using dynamic light scattering (Zetasizer Nano ZS series, Malvern Instruments, Worcestershire, UK). Single and clustered o/w emulsions were diluted at least 100x with the corresponding aqueous phase (without emulsifier). Each emulsion was measured in triplicates at 20°C.

### Mechanical properties

Fracture stress, fracture strain and Young's modulus of the gels were determined with a Texture Analyser (TA.XT plus, Stable Micro Systems-SMS, equipped with a 50 kg load cell). The cylindrical gel specimens had a diameter of 23.1 mm and a height of 10 mm. The gels were compressed with a circular aluminium probe with a radius of 50 mm. Both plate and top of the gel surface were lubricated with a thin layer of paraffin oil to prevent friction between plate and sample during compression. Fracture measurements were performed by applying compression at room temperature at a constant compression speed of 2 mm/s up to a compression strain of 80%. Nine replicates (3 pieces per syringe and three syringes per type of gel, each prepared separately) were measured, and the mean values for true fracture stress, true fracture strain and Young's modulus were calculated.

The experimental values of Young's modulus were compared with values calculated according to the Kerner model and a modification of it. The Young's modulus of the homogeneous gels was calculated using the Kerner model (Kerner, 1956). To estimate the modulus of the gel-in-gels, a modified Kerner model (Equation 1) was used, which was introduced by van Aken et al. (2015) for gels with an inhomogeneous droplet distribution. The relative modulus Er (modulus of the mixed gel relative to the modulus of the matrix) is related to the Poisson ratio of the oil droplets, $v_m$, the volume fraction, $\varphi$, the ratio between the moduli of the particles and the outer phase, $M$, and the oil volume fraction ratio between the filler and the continuous phase, $\chi$ as

$$E_r = \frac{15 * (1 - v_m)(M - 1) * \chi * \varphi_{total}}{(8 - 10 * v_m) * M + 7 - 5 * v_m - (8 - 10 * v_m) * (M - 1) * \chi * \varphi_{total}} + 1 \qquad 1$$

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We assumed $v_m$ = 0.5 (van Aken et al., 2015). More details of this model can be found in the Appendix as supplementary information. Oil concentrations were converted to oil volume fractions.

### 4.2.6 Sensory evaluation

For the sensory evaluation of all gels, the "Rate All That Apply" method (RATA) was used with untrained participants (Meyners, Jaeger, & Ares, 2016). Participants (n"gel-in-gel"=50, n"hetero-aggregates"=56, different subjects in both groups) were mostly students recruited from Wageningen University & Research Campus (average age "gel-in-gel": 23.9 ± 3.1 years, 71% female, 29% male; average age "hetero-aggregates": 23.0 ± 2.8 years, 66% female, 34% male). Participants were selected based on their absence of any intolerance or allergy towards the ingredients present in the samples and upon confirmation of general health by the participant. The participants received financial compensation upon completion of the study.

The sensory testing was performed in meeting rooms of Wageningen University. Separators were used to create booths to prevent communication between participants. The participants evaluated the samples in randomised order. Gel-in-gels and emulsion-filled gels with hetero-aggregates were assessed in separate sessions of 60 minutes each. During each session, participants evaluated 3 or 4 samples with breaks of 2 minutes between samples, in which participants were asked to rinse their mouth with water. The samples were presented as gel cylinders (23.1 mm diameter and 10 mm height) at room temperature, in paper cups coded with a random three-digit code. Participants were asked to perform a tablet-based "Rate-All-That-Apply" (RATA) questionnaire with a given list of selected terms (Table 3), using the EyeQuestion software (V. 4.11). Attributes were rated using a discrete scale from 1-9 with anchors weak and strong, and the possibility to select "not applicable" (taken as "0"). The attribute selection and definition is based on the work of Oppermann et al. (2017); van Aken et al. (2011) and Benjamins et al. (2009). Attribute definitions were adjusted when required, *e.g.* in cases of food product indications. Between each sample set, participants were asked to rinse their mouth with water.

The research of this study does not fall within the remit of the 'Medical Research Involving Human Subjects Act'. The study was conducted in agreement with the ethical principles regarding human experimentation outlined in the Declaration of Helsinki. Participants gave written informed consent.

**Table 3. Sensory attributes provided to participants for RATA evaluation, including descriptions. Attributes marked with an asterisk * were only used for hetero-aggregate containing gels.**

| Sensory attribute | Description |
|---|---|
| Graininess | A product contains grains. Feeling like particles remain in the mouth, can be small or big. |
| Creaminess | The intensity of creaminess/softness. Creaminess is a soft,full feeling in the mouth, which is also thick-soft and supple. The product is not rough and not dry. It leaves a soft, fatty feeling. It is perceived in the whole mouth and gives a velvety feeling in the whole mouth. |
| Stickiness | The intensity of stickiness. Stickiness describes a sticky feeling that can be perceived with tongue and palate. An example of a sticky product is a caramel candy bar. It is perceived between the teeth during a chewing movement. |
| Homogeneity | A product is homogeneous if it contains no particles or other parts that could be felt while chewing. |
| Oiliness | Oiliness is the intensity of fatty/oily feeling in the mouth. It gives a smooth feeling and a coating on the palate. An oily/fatty layer that stays in the mouth. |
| Wateriness | Consisting of containing water, thin, weak texture, opposite of viscous, lacking body |
| Hardness | The intensity of hardness of the product in the mouth when taking a bite. |
| Coating | The degree to which a product leaves a coating feeling in the whole mouth. It can be felt on the teeth and the palate. |
| Lingering | A feeling that the sample or perception of the sample stays long in the mouth. |
| Melting | Sample melts during chewing |
| Brittleness* | A brittle sample breaks easily (dry cookie), the opposite is elastic |
| Oil flavour | The intensity of oily, fatty flavour. Taste of salad oil or fat. |
| Sweetness | The intensity of sweet flavour. Sweet taste. |
| Sourness* | Perception of sourness |
| Bitterness* | Perception of bitter taste |

### 4.2.7 Statistical data analysis

The statistical data analysis was performed using RStudio Version 1.1.423 (RStudio Inc.) and R 3.4.3, with the additional packages agricolae, dplyr and FactoMineR. For the RATA sensory data, we found that the data were not normally distributed. Therefore, we analysed the RATA data using a non-parametric Friedman test and Fisher's least significant differences. This implies that we transformed the data from RATA intensity values to ranks. This transformation preserves the relative position of the different samples within one attribute; however, the information on the intensity of the rating is lost. The results of the RATA test are expressed as the sum of ranks for each sample for each attribute. This procedure has been suggested previously by Meyners et al. (2016) for RATA data analysis. Differences among other characterisation parameters (*e.g.* particle size and mechanical properties) were evaluated using an ANOVA and a Tukey post-test. For all statistical data analysis, $p < 0.05$ was chosen as the significance level.

## 4.3. Results and discussion

### 4.3.1 Effect of oil droplet clustering at a μm-scale

Microstructure and cluster size

Hetero-aggregated emulsions stabilised with gelatine and DATEM were incorporated in the low acyl gellan gel matrix. For comparison, also gels in which the non-clustered emulsions were homogeneously distributed were prepared. In the case of the pre-clustered emulsions, both small (GD28) and large (GD55) clusters remained mostly intact after incorporation into the gel. We confirmed that the visible clusters consisted of individual oil droplets by taking CLSM images of the samples in which the oil was stained with Nile Red (Figure 1).
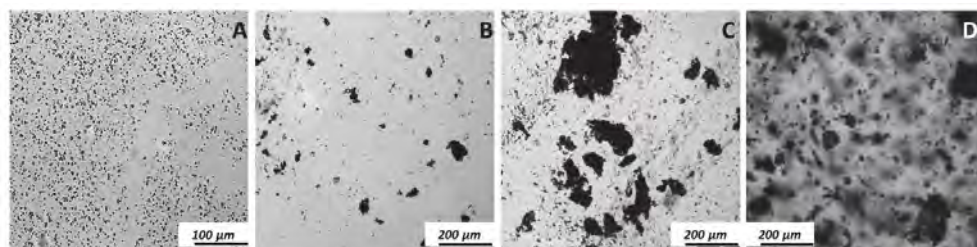


**Figure 1. Micrographs of low acyl gellan gels with A) gelatine-stabilised emulsion, B) GD28 emulsion with small clusters, C) and D) clustered emulsion with large clusters (GD55). Samples A-C are light microscopy images. Sample D is a CLSM image. In sample D, the oil phase was stained with Nile Red. Scale bars refer to a size of (A) 100 μm or (B, C, D) 200 μm. The oil volume fraction was reduced to 1% for sufficient cluster separation.**

This observation is relevant as only in the case of clusters with individual droplets, contrary to coalesced droplets, an increase in effective volume fraction and entrapment of continuous phase can be expected. This entrapment and increase in effective volume fraction can consequently affect mechanical properties, and, potentially, perception. In Figure 1D, it can be seen that also large clusters consisted of individual droplets, although the images cannot provide quantitative information on the characteristics of the clusters. However, after incorporation of the clusters in a gel, the cluster size was smaller compared to that of the oil droplet clusters in liquid o/w systems. For example, large clusters (GD55) in the gel had an average effective diameter of 20.5 ± 5.2 μm, which was smaller than the 50 μm found for the cluster size

in the o/w emulsions; the gelation process decreased the size of the clusters. The observed reduction in the size of the large clusters could potentially be explained by three reasons. Firstly, the competition of the negatively-charged oil droplets and low acyl gellan gel for electrostatic attraction with the positively-charged gelatin-stabilised oil droplets might induce a partial disaggregation of the oil droplets clusters. Secondly, a screening of the charges by the salt present in the gel (150 mM NaCl) might limit cluster (re-)formation by reduction of the electrostatic attraction between oppositely-charged emulsion droplets. Thirdly, the shear applied during incorporation of clusters into the gels may contribute to the disruption of the clusters.

Small clusters (GD28) did not change noticeably in size when incorporated into the gel. Gels containing GD28 clusters showed an average size of 10.4 ± 1.7 μm, which was comparable to the size of the clusters when present in the o/w emulsions (8 μm). We suggest that the small clusters (GD28) quickly reform. When WPI and gelatine were used as emulsifiers to prepare hetero-aggregated clusters, no clusters were present in the gels anymore. In this case, the clusters were shear-sensitive, independently of their initial size, and the low stability of these clustered systems led to their disintegration during gel formation. This observation confirmed that electrostatic interactions are of great importance in the stabilisation of the clusters, and essential for successful incorporation of the clusters into a semi-solid, gel matrix. As a consequence of the low stability of WPI-gelatine stabilised clusters, no gels with WPI-gelatine hetero-aggregates could be included in this study.

To summarise, hetero-aggregated o/w emulsions can be incorporated into a continuous gel matrix to obtain emulsion-filled gels with clustered oil droplets. However, to control the extent of the inhomogeneity is challenging and depends mainly on the set of emulsifiers used and the processing conditions.

Mechanical properties

An overview of the mechanical properties of the low acyl gellan gels can be found in Table 4. We observed that the incorporation of non-clustered o/w emulsions affected the Young's modulus of the gels significantly. The modulus of gels with a homogeneous distribution of gelatine-stabilised oil droplets increased by 30%. As gelatine-stabilised droplets can be considered bound fillers, based on attractive electrostatic interactions between droplets and matrix, and the droplets are stiffer than the matrix (modulus matrix: 66 kPa; modulus droplets: ~240 kPa (see Appendix A)), the modulus of the gelatine-stabilised emulsion-filled gel increases. This gel reinforcement by bound fillers was confirmed previously for various types of gels, including polymer gels (gelatine) and particle gels (WPI) (Sala et al., 2007a; van Vliet, 1988). In the case of gels with DATEM-stabilized emulsions, the modulus decreased by 12% compared to the matrix. As DATEM-stabilised droplets carry the same charge as the matrix, they are not incorporated into the matrix and should be considered as unbound droplets; consequently, the modulus of the filled gel decreases, which has been discussed before by other authors (Chen & Dickinson, 1998).

**Table 4. Mechanical properties of gels with incorporated (hetero-aggregated) oil droplet clusters. *This value was obtained from literature (Cassanelli, Prosapio, Norton, & Mills, 2018). Different letters indicate significant differences between values (p<0.05).**

| Sample | Label | Emulsifier | Average droplet/cluster size [µm] | Zeta potential [mV] | Young's modulus [kPa] | Relative modulus $E_r$ [-] | True fracture stress [kPa] | True fracture strain [-] |
|---|---|---|---|---|---|---|---|---|
| Matrix | - | - | - | -20* | 66.0 ± 1.3[a] | 1.00 | 32.2 ± 1.9[a] | 0.35 ± 0.03[a] |
| Homogeneous distribution | GD10 | gelatine | 2.5 ± 1.0[a] | 10.0 ± 0.2[a] | 86.2 ± 3.7[b] | 1.30 | 32.9 ± 1.0[a] | 0.38 ± 0.02[a] |
| Homogeneous distribution | GD01 | DATEM | 2.0 ± 1.3[a] | -70.2 ± 0.6[b] | 57.9 ± 5.1[c] | 0.88 | 11.2 ± 4.2[b] | 0.20 ± 0.01[b] |
| Small clusters | GD28 | gelatine/DATEM | 10.4 ± 1.7[b] | -26.3 ± 1.4[c] | 89.9 ± 2.2[b] | 1.34 | 21.9 ± 0.6[c] | 0.23 ± 0.05[bc] |
| Large clusters | GD55 | gelatine/DATEM | 20.0 ± 5.2[c] | -1.6 ± 1.5[d] | 105.0 ± 4.3[d] | 1.59 | 27.9 ± 1.6[d] | 0.27 ± 0.03[c] |

The clustering of the oil droplets in the gels strongly affected the mechanical properties of the emulsion-filled gels. Upon incorporation of small oil droplet clusters (GD28) into the gel matrix, the Young's modulus of the gel increased by more than 30% (Table 4), indicating that the clusters were most likely incorporated into the matrix. Even though the clusters displayed an overall negative charge of -26 mV (Table 4), positive charges could be present as patches within the cluster, consequently allowing the incorporation or the partial incorporation of clusters. An additional aspect that might contribute to the stiffening of the material is the anisotropy of clusters. Furthermore, in our model systems complexes between free gelatine and DATEM might have formed at the oil droplet interface and contribute to the network strengthening. Such an effect of complexes was discussed previously by Hong and Dickinson (1996), who suggested that for emulsion-filled protein gels with β-lactoglobulin and DATEM, protein-surfactant complexes formed at the interface could contribute to the network strength. In the case of large clusters (GD55), with a very low zeta potential of -1.6 mV (Table 4), the change in Young's modulus was even further pronounced. As the charge was low, the droplets were most probably incorporated into the gel. The significant degree of clustering increased the modulus by 60%. This result is comparable to previously published data on clustering of oil droplets. Oliver et al. (2015a) showed that clustering of WPI-stabilised oil droplets in gelatine gels, clustered by adjusting gelation kinetics, leads to an increase in Young's modulus of around 50%. A reason for the increase in Young's modulus upon droplet clustering lies in the increase in the effective volume fraction of the oil droplets. The clustering reduces the free (aqueous) bulk phase and thereby effectively increases the volume fraction of the dispersed (oil) phase. It is of interest to compare this result to findings in liquid o/w emulsions, where we have shown that the effective oil volume fraction of clusters in liquid o/w emulsions can increase by a factor of up to 5x (Fuhrmann et al., 2019a). We suggest that an increase of the effective volume fraction also occurs when clustered emulsions are incorporated into emulsion-filled gels.

Both the true fracture stress and strain were strongly affected by the type of emulsifier used to stabilise the oil droplets than by clustering. When gelatine-stabilised emulsion droplets were incorporated into the matrix, the true fracture stress remained mostly unaffected. When DATEM-stabilized oil droplets were present, the true fracture stress decreased significantly. The difference can be attributed to the charge of the emulsifiers used. DATEM and low acyl gellan carry both a negative charge; thus, the droplets do not adhere to the matrix (Cassanelli et al., 2018). Due to electrostatic repulsion between the droplets and the matrix, fracture events might occur more quickly, which reduces the fracture stress (Nielsen, 1966). Similar conclusions were drawn by Sala et al. (2009) for the incorporation of unbound fillers (Tween-stabilised oil droplets) into polymer gels (*e.g.* gelatine). The fracture stress was not strongly influenced by the clustering of the oil droplets. Gels with large clusters displayed a fracture stress between that found for the gels containing gelatine-(GD10)- and DATEM-(GD01)-stabilised oil droplets only. Emulsion-filled gels containing

small clusters (GD28) had a true fracture stress more similar to that of the DATEM-stabilised emulsions, due to a higher fraction of DATEM-stabilised droplets in the cluster. These findings suggest that the fracture behaviour of the emulsion-filled gels is strongly affected by the interaction between emulsifier and matrix, rather than by the clustering.

Also, the true strain of the gels depended on the type of emulsifier used. Gels with DATEM-stabilised emulsions became considerably more brittle. The decrease in the fracture strain of the DATEM-stabilised droplets can be related to the fact that the droplets are unbound due to the electrostatic repulsion between the droplets and the continuous phase. The network structure might be disrupted by the DATEM-stabilised droplets, and a more brittle gel is formed. Gels filled with gelatine-stabilised emulsions (GD10) had a fracture strain similar to the unfilled gels. Gelatine-stabilised droplets act as bound fillers. From previous research, a decrease in fracture strain could be expected upon the incorporation of bound fillers in a gel (Sala, van Aken, Stuart, & van de Velde, 2007a). However, the fracture strain of the gel, upon the incorporation of gelatine-stabilised droplets, was not notably affected. Therefore, a different phenomenon had to contribute to the observed fracture behaviour. One possible explanation is the interaction between the gelling agent (gellan) and free surfactant (gelatine). Previous research has shown synergistic effects in the gelling behaviour between mixed gels of gellan and gelatine, with higher elasticity of mixed gellan-gelatine gels, compared to gellan gels alone (Papageorgiou & Kasapis, 1995; Papageorgiou, Kasapis, & Richardson, 1994). We hypothesise that small amounts of gelatine in the aqueous bulk phase of the incorporated emulsions interacted with the gellan within the network. This could result in a reduction of the brittleness of the gel. To test this hypothesis, we created a series of gellan gels to which we added an increasing amount of gelatine. The results indicated that already a small amount of gelatine (0.125% (w/w) in the final mixed gel) increased the fracture strain by 20%, thus made the gel more elastic (results not included). We, therefore, suggest, that the limited change in fracture strain by the incorporation of the gelatine-stabilised droplets is due to a combination of two opposite effects: firstly, the fracture strain increases due to incorporation of gelatine into the gellan network, and secondly, it decreases due to the incorporation of bound fillers, compared to a non-filled gel. Clustering influenced the fracture strain, similarly to the fracture stress, only to a minor extent.

To summarise, the presence of inhomogeneity in the oil droplet distribution at the μm-scale of the emulsion-filled gels influenced the Young's modulus leading to stiffer gels with increasing cluster size. Fracture properties were influenced by the interactions between the oil droplets and the matrix rather than clustering.

### Sensory properties

As mentioned in section 4.2.7, the RATA intensity data were transformed into rank orders and analysed using non-parametric Friedman tests. The RATA data are, therefore reported as the sum of ranks for each sample and for each attribute (Meyners et al., 2016). Different emulsifiers, as well as the clustering of oil droplets, changed the sensory perception of emulsion-filled gels. Hardness, creaminess, graininess, melting, and oil flavour perception differed significantly among gels (Figure 2). An overview of the gels tested can be found in Tables 1 and 4.
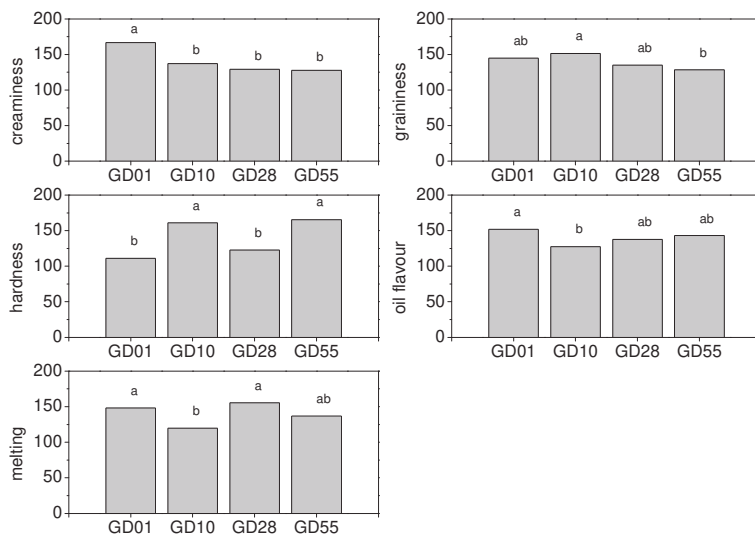
**Figure 2. Rank sum of attributes of emulsion-filled gels with DATEM-stabilised emulsions (GD01), gelatine-stabilised emulsions ( GD10), emulsions with small clusters (GD28) and large clusters (GD55). Only attributes with significant differences (p<0.05) among samples are shown. The RATA intensity data was transformed into rank orders and analysed using a non-parametric Friedman test.**

In terms of texture-related attributes, we observed that especially perceived hardness varied among samples based on the interaction between the droplets and the matrix, the size of the clusters, and the results were in line with the measured fracture stress and Young's modulus. Emulsion-filled gels containing large clusters (GD55) and gels with gelatine-stabilised droplets (GD10) were perceived as the hardest, which is consistent with a higher Young's modulus and fracture stress. Gels with lower Young's modulus and fracture stress, such as DATEM-stabilised droplets (GD01), were perceived as the least hard.

In terms of fat-related attributes, creaminess was related to the type of emulsifier rather than cluster size. Gels containing non-clustered DATEM-stabilised oil droplets were perceived as more creamy compared to gels with non-clustered gelatine-stabilised oil droplets. Previous research has shown that the perception of fat-related attributes depends on three parameters: (i) whether droplets are bound or unbound (Liu et al., 2015), (ii) the fracture properties of the mixed gel (Sala et al., 2008) and (iii) the melting behaviour of the matrix (Sarkar, Ye, et al., 2017).

In the gel containing DATEM-stabilised droplets (GD01), the droplets were unbound, while gelatine-stabilised droplets (GD10) were bound to the matrix. Gels with a homogeneous distribution of gelatine-stabilised droplets (GD10) showed rather low creaminess. We suggest that the bound droplets were hindered in their release and, consequently, creaminess was ranked low for this gel (GD10). Accordingly, the DATEM-stabilised droplets (GD01) were released more easily from the matrix and thus were perceived more creamy. Similar findings were reported by Liu et al. (2015) for emulsion-filled gelatine gels with unbound emulsion droplets, showing higher creaminess for gels with unbound droplets. We, furthermore,

observed in our study that more brittle gels were perceived as more creamy. Such a relation between
fracture properties and perception was discussed by Sarkar et al. (2017), who showed that gels with a low
fracture strain (so more brittle gels) tend to release more oil than elastic gels. This is similar to our findings
in this study. However, also the opposite phenomenon has been reported, in a study that showed that
emulsion-filled gels with higher fracture strain were perceived as more creamy ( Devezeaux de Lavergne,
Strijbosch, van den Broek, van de Velde, & Stieger, 2016). The differences in results between studies might
arise, for example, from different gelling agents used in the different studies (gellan was used in this study,
whereas agar/gelatine and a combination of kappa-carrageenan/locust bean gum and gellan were used in
the other studies). Also, the melting properties may influence the results of different studies. As we used
a non-melting polymer in this study, the melting behaviour of the gelling agent could not influence the fat-
related perception.

The degree of release of the oil droplets is also in line with the observations obtained for the attribute "oil-
flavour". This attribute was ranked highest for GD01 (DATEM-stabilised), while it was lowest for GD10
(gelatine-stabilised). Gels with clusters (GD55 and GD28) also show lower values for creaminess and
comparable values for oil-flavour. Similarly to the gelatine-stabilised droplets, the clusters were most
probably bound to the matrix, which hindered their release.

Furthermore, the release of droplets and the fracture properties also seem to play a role in the melting
perception. Differences in melting sensation in these gels could not arise from an actual melting of the gels
since gellan does not melt in the mouth. Melting perception was low in the gels with higher amounts of
gelatine-stabilised emulsions and higher in gels containing either DATEM-stabilised emulsions or
emulsions with gelatine-DATEM clusters; the melting perception was thus higher for gels with unbound
droplets. However, as gels with small clusters (GD28) had the highest melting scores, also the cluster size
influenced melting perception. Melting perception also seems to be associated with changes in the
fracture strain of the gels. This hypothesis is in line with literature; gels with a rather low fracture strain
have been reported to elicit a high melting perception, whereas samples with high fracture strain show a
lower rating for the attribute melting (Sarkar, Ye, et al., 2017).

Our previous study (Fuhrmann et al., 2019b) showed that in liquid o/w emulsions, large, strongly-
interacting oil droplet clusters could be perceived as grainy. When incorporating these strongly-interacting
clusters in a semi-solid emulsion-filled gel, the perceived graininess decreased compared to homogeneous
emulsion-filled gels. We suggest that this is related to two effects. Firstly, the graininess might be masked
by the gel. It has been previously described that the perception of graininess can depend very much on
the matrix properties, such as hardness or viscosity (Imai, Hatae, & Shimada, 1995). Secondly, the observed
reduction in graininess could also be related to lubrication effects. As clustered oil droplets have shown to
reduce friction (Fuhrmann et al., 2019b), the improved lubrication might have reduced the grainy
perception of the gel. These findings, however, might depend on matrix type and concentration; in
emulsion-filled gels with lower gelling agent concentration, the graininess might not be masked.

Overall, it was shown that the interactions between emulsifier and matrix dominate the mechanical properties of the gels. The clustering of the oil droplets itself, at this length scale, had a limited effect on fat-related sensory perception, but rather on texture attributes.

### 4.3.2 Effect of oil droplet inhomogeneity at a mm-scale

Physical properties

To evaluate how oil droplet inhomogeneity at mm-scale affects mechanical and sensorial properties, we developed an experimental approach based on the incorporation of emulsion-filled gel particles in an emulsion-filled gel matrix. An overview of the mechanical properties of the gel-in-gels can be found in Table 5.

**Table 5. Mechanical properties of homogeneous gels and gel-in-gels.*relative Modulus $E_r$ refers here to the modulus of the gel, relative to the matrix modulus. ‡relative modulus $E_r$ refers here to the modulus of the overall gel, relative to the modulus of the continuous phase. Predicted modulus was calculated acc. to Eq. 1. Different letters indicate significant differences between values (p<0.05).**

| Distribution | Label | Oil concentration (in) | | | Young's modulus [kPa] | Relative modulus $E_r$ [-] | Predicted Young's modulus [kPa] | True fracture stress [kPa] | True fracture strain [-] |
|---|---|---|---|---|---|---|---|---|---|
| | | Total [%] | Particles [%] | Continuous phase [%] | | | | | |
| Homogeneous gelatine gels | - | 5 | | 5 | 6.1 ± 2.0[a] | 1.0[*] | 6.5 | 13.0 ± 3.2[bc] | 1.3 ± 0.1[a] |
| | | 17.5 | | 17.5 | 8.0 ± 2.3[a] | 1.4[*] | 8.5 | 15.7 ± 2.7[ab] | 1.1 ± 0.1[b] |
| | | 30 | | 30 | 16.9 ± 3.3[b] | 2.9[*] | 11.1 | 17.9 ± 2.8[a] | 0.9 ± 0.1[c] |
| "Gel-in-gel" samples | 30/5 | 17.5 | 30 | 5 | 7.9 ± 2.2[a] | 0.5[‡] | 8.4 | 9.7 ± 2.8[c] | 0.9 ± 0.1[c] |
| | 17.5/17.5 | 17.5 | 17.5 | 17.5 | 8.3 ± 1.5[a] | 1.0[‡] | 8.5 | 9.7 ± 2.8[c] | 0.9 ± 0.1[c] |
| | 5/30 | 17.5 | 5 | 30 | 7.9 ± 2.3[a] | 1.3[‡] | 8.5 | 9.3 ± 3.1[c] | 1.0 ± 0.1[bc] |

Firstly, we discuss the mechanical properties of the particles and the individual gels. By incorporating oil droplets into the gelatine matrix, the Young's modulus of the gelatine gels increased with increasing oil concentration. The used gelatine had a slight net positive charge at pH 7, while WPI, used for stabilising the oil droplets (pI 5), had a net negative charge at the same pH. Thus, attractive electrostatic interactions between emulsion droplets and the matrix occurred.

Consequently, the oil droplets acted as bound fillers. As the droplets were more than 10 times stiffer than the matrix, (about 100 kPa for the droplets, see Appendix A), the modulus increased with filler content. Using the Kerner equation (Kerner, 1956), the modulus of the homogeneous, emulsion-filled gels can be predicted. The calculated moduli for homogeneous emulsion-filled gelatine gels were 6.5, 8.5 and 11.1 kPa for samples with an oil volume fraction of 0.05, 0.175 and 0.3. These values were in good agreement with the measured values of 6.1, 8 and 16.9 kPa (Table 5).

The fracture stress did not vary significantly (p>0.05) for gelatine gels containing 5% and 17.5% oil, stabilised with WPI, in line with experiments by Sala et al. (2007a). However, it increased slightly (p<0.05), from about 13 kPa (5% oil) to 18 kPa, for a 30% oil concentration, so the relative fracture stress σr was 1.38. This increase in fracture stress is also predicted, yet slightly underestimated, by a theoretical model proposed by Langley and Green for bound fillers (Equation 2) (Langley & Green, 1989). In this model the relative fracture stress, $\sigma_r$, is related to the volume fraction, φ, of the fillers as

$$\sigma_r = \frac{\left(1 - \frac{\varphi^{\frac{1}{3}}}{\varphi_g}\right)}{\left(1 - \frac{\varphi}{\varphi_g}\right)^{\frac{5}{2}}}$$

$\qquad\qquad$ 2

where the oil droplets are the fillers, and $\varphi_g$ is the maximum packing volume, which was taken as 0.64 for random close packing. This model predicts a relative stress of 1.09, which is lower than the measured value of 1.38. The deviation might be explained by the type of the network. The model was developed for particle gels, whereas gelatine behaves more as a polymer gel.

In addition, the interactions between the droplets and the matrix may play a role. Further investigations on this model are, however, out of the scope of this research.

In terms of fracture strain, we observed a strong effect of the incorporation of oil into the gel on this parameter. The incorporation of an increasing amount of oil led to a decrease in fracture strain, and the gel became more brittle, which is in line with literature (Sala, van Aken, et al., 2007a).

*Incorporation of particles to create gel-in-gels*
When incorporating oil-containing particles in a matrix with the same oil concentration (17.5/17.5), the samples displayed a Young's modulus similar to that of a homogeneous sample with 17.5% (w/v) oil. The incorporation of particles with the same mechanical properties did not change the gel stiffness. Consequently, noticeable differences for the other gels can thus be attributed to the distribution of the oil droplets. In the case of samples with an inhomogeneous droplet distribution (5/30 and 30/5), the Young's modulus was also very similar to the one of the homogeneous sample with an overall oil content of 17.5% (Table 5). This result suggests that for large scale (mm) inhomogeneity of oil droplets, the moduli are not affected; the overall oil concentration is more important than local concentration differences (mm-scale inhomogeneity).

The fracture properties of the gels were affected by the incorporation of particles, rather than by the differences in oil droplet distribution. For homogeneously filled gels, the fracture stress increased from 13.0 to 17.9 kPa, and the fracture strain decreased from 1.3 to 0.9, with an increase in oil content from 5% to 30%. A strong decrease in fracture strain was shown previously for polymer gels reinforced by oil droplets (*e.g.* WPI-stabilised oil droplets in gelatine) (Sala et al. 2007a).

For all gels with an inhomogeneous oil droplet distribution, the true fracture stress decreased to about 9 kPa after incorporation of particles. The true fracture strain was also affected by the incorporation of the particles. For all inhomogeneous gels with 17.5% oil in total, the fracture strain was between 0.9 and 1.0 (not significantly different), and therefore resembled more the fracture strain of the filled gel with 30% oil content. Incorporation of particles with a higher modulus/stress and higher brittleness (30%) into a matrix with the lower modulus (5%) thus increases the brittleness of the gel. The incorporation of particles with a lower modulus, yet lower brittleness (5%), into a matrix with a higher modulus and higher brittleness (30%) does not lead to significant changes in the brittleness, so a change in deformability or elasticity. This result is in line with literature (Brownsey, Ellis, Ridout, & Ring, 1987; Sala, Van Aken, et al., 2007a).

To conclude, the Young's modulus was affected by the total oil volume fraction of the gel-in-gels, and not by an inhomogeneous distribution of oil droplets on mm-scale. The fracture properties of gel-in-gels were slightly affected by the droplet distribution, but the differences were rather small. Droplet inhomogeneity at mm-scale in gel-in-gels has a limited effect on mechanical properties.

Sensory properties

As mentioned in section 4.2.7, the RATA intensity data were transformed into rank orders and analysed using a non-parametric Friedman test. The RATA data was therefore reported as the sum of ranks for each sample for each attribute (Meyners et al. 2016). An overview of the attributes that were perceived as significantly different among the gel-in-gels is given in Figure 3. Hardness, oiliness, oil flavour, homogeneity, lingering, creaminess, graininess, stickiness and mouth-coating differed (p<0.05) among the gel-in-gels.
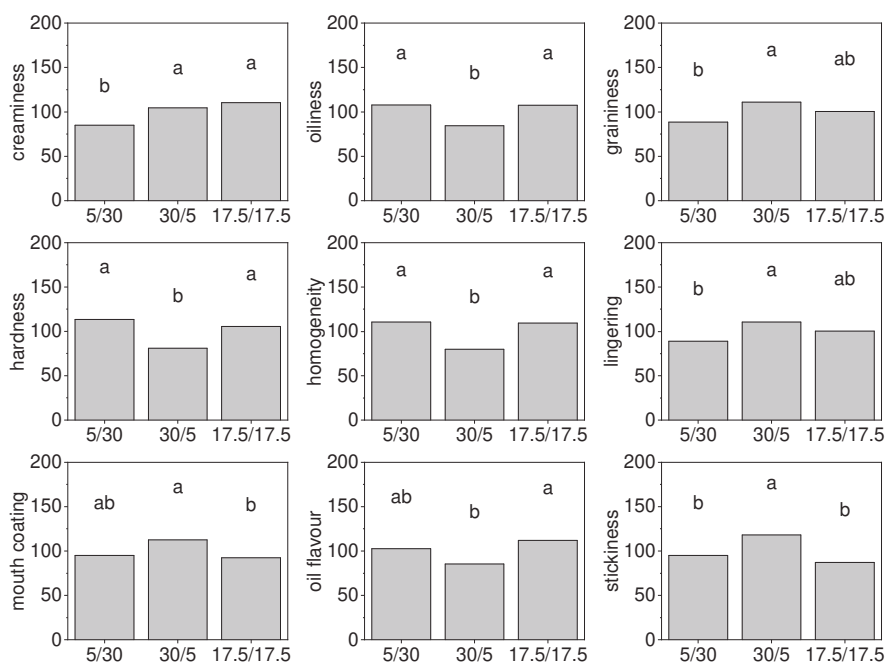


**Figure 3. Rank sum of attributes of gel-in-gels. Gel-in-gels with 5% (v/v) oil in the particles and 30% (v/v) oil in the continuous phase are denoted as 5/30, gel-in-gels with 30% (v/v) oil in the particles and 5% (v/v) oil in the continuous phase as 30/5, and gel-in-gels with 17.5% (v/v) oil in both the particles and the continuous phase are abbreviated as 17.5/17.5. Only attributes with significant differences (p<0.05) among samples are shown. The RATA intensity data was transformed into rank orders and analysed using non-parametric Friedman tests.**

In terms of texture attributes, mainly hardness, homogeneity and graininess perception changed for the different samples. The perceived hardness was lower in the gel-in-gels with a lower oil concentration in the continuous phase (30/5). This sample had a lower modulus and fracture stress in the continuous phase than in the dispersed particles. As the gel-in-gels were overall comparable in Young's modulus and fracture

stress independently of the droplet distribution, this finding suggests that the perceived hardness is dominated by the fracture stress and modulus of the continuous gel matrix.

The oil droplet distribution also affected perceived homogeneity and graininess. Homogeneity was lowest in gel-in-gel 30/5, containing particles with the highest individual fracture stress. A high homogeneity was found to be related to the continuous phase with high fracture stress. Graininess was ranked highest in gel-in-gel 30/5, thus, in gels containing gel particles with the highest individual fracture stress.

In terms of fat-related attributes, oiliness and oil flavour were primarily influenced by the continuous phase; the gel-in-gel (30/5) with the lowest oil concentration (5% (v/v)) in the continuous phase scored lowest for these attributes. In contrast, gel-in-gels with higher oil concentration in the continuous phase scored higher (17.5/17.5 and 5/30). This observation can be linked to the fact that more oil droplets can reach oral surfaces; higher oil concentration thus leads to higher oiliness and oil flavour. Similar findings have been reported for various gel types, including carrageenan, gelatine and WPI gels (Sala, van de Velde, et al., 2007). Unexpectedly, gel-in-gels containing the lowest oil concentration in the continuous phase were perceived as more mouth coating and more lingering. These attributes, therefore, seem not to be determined by oil release solely and could be related to melting events. From previous research, we see that the melting point of emulsion-filled gelatine gels can change upon oil incorporation. Hattrem and co-workers (2015) showed that for gelatine (type A, as used in this experiment), the melting temperature could increase remarkably, by more than 10°C, with an increasing amount of incorporated oil. Gels with high oil content, therefore, melt later or not at all during consumption. Thus, the gel-in-gel 30/5, with a continuous phase containing a low amount of 5% (v/v) oil, might have melted faster during oral processing, compared to gel-in-gels with higher oil concentration (17.5 and 30% (v/v)). The faster melting could have increased coating and lingering perception. Thus, we found that the mechanical properties alone are not sufficient to explain these differences, as they were found to be very similar between the samples.

In line with the differences in the melting temperature, stickiness was highest for samples with low oil volume fraction in the continuous phase; a lower melting temperature might facilitate the formation of a layer of the molten matrix around the sample and on the oral surfaces and increase perceived stickiness.

Surprisingly, the creaminess of gel-in-gels with a high oil concentration in the continuous phase (5/30) was ranked lower than that of gel-in-gels with a lower oil concentration (30/5 and 17.5/17.5) in the continuous phase. This finding contrasts with the results reported by Mosca et al. (2012). In their work on inhomogeneous fat distribution on mm-scale, obtained by layering different gels, creaminess tended to increase with increasing inhomogeneity of the oil droplet distribution. We did not observe an increase in creaminess with an inhomogeneous distribution. A possible reason for the lower creaminess in our results could again be related to the change in the melting temperature of the continuous gel phase. Creaminess perception has been shown to depend on the formation of a coating layer in the mouth (Dickinson, 2018). Therefore, if the oil content and the accompanying melting temperature increase, the formation of a coating layer would be reduced, and perceived creaminess could have decreased.

These results demonstrate that the incorporation of an inhomogeneous oil droplet distribution at mm-scale obtained with a gel-in-gel approach influences sensory perception considerably. The inhomogeneity

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of the oil droplet distribution can change texture-related attributes such as hardness, but also fat-related sensations, such as oiliness and coating perception.

### 4.3.3 Effect of the length scale of inhomogeneity of oil droplets on mechanical and sensory properties: hetero-aggregation vs gel-in-gels

When comparing the two approaches to obtain inhomogeneity in the oil droplet distribution in emulsion-filled gels, one must be careful with generalisations, as it must be considered that different gelling agents were used to prepare gels with inhomogeneities at µm and mm-scales. As previously mentioned, different gelling agents will influence the fracture behaviour and the accompanying sensory perception. The reason for this decision was of a practical nature. The use of gelatine as a matrix for the clustered samples led to a reduction in the cluster size (not shown), and therefore gellan was used as the preferred gelling agent. For the gel-in-gels, gelatine was the preferred choice, as it easily incorporates the gelatine particles forming a cohesive gel. Consequently, a direct comparison of the results between the two gels is difficult. Nonetheless, the findings confirm that an interplay of emulsifier-matrix interactions and droplet distribution influence the mechanical and sensorial properties of food gels. The length scale of the inhomogeneity determines whether the mechanical properties and texture attributes or fat-related sensory attributes are affected. Mechanical properties are relevant to the texture perception. By increasing the modulus and fracture stress, the perceived hardness of an emulsion-filled gel can increase. This increase can be achieved by either modifying droplet-matrix interactions, thus creating an active filler, or by clustering oil droplets on a small length scale. Large scale oil droplet inhomogeneity is less suited to modify texture properties such as hardness. However, our findings highlight the influence of the mechanical properties of the continuous matrix on perceived hardness of an emulsion-filled gel. Based on literature, we stress that this finding will depend on overall polymer type and concentration (Foegeding, 2007).

In terms of fat-related attributes, large scale oil droplet inhomogeneity (mm scale) is more efficient to modify sensory perception than small scale oil droplet inhomogeneity. With large scale inhomogeneity, oiliness and oil flavour perception can be tuned. Both attributes were reduced with decreasing the oil concentration of the continuous gel phase, while creaminess perception stayed constant. Mouth coating, lingering perception, and stickiness increased for lower oil concentrations in the continuous phase.

The large-scale inhomogeneity might also lead to a grainy perception. In case the oil concentration of the dispersed particles is higher, the particles become stiffer and therefore might be perceivable. Such graininess perception is not observed with µm-scale inhomogeneity, most likely because the clusters are too small to be perceived.

### 4.4. Conclusions

This study aimed to determine the effect of inhomogeneity of oil droplet distribution at different length scales on mechanical and sensory properties of semi-solid gels. We showed that controlled inhomogeneous distribution of oil droplets in a semi-solid gel was achieved at two length-scales, namely on the µm-scale and on the mm-scale.

On a μm-scale, oil droplet inhomogeneity by droplet clustering influenced mostly the mechanical properties of the emulsion-filled gels. With respect to sensory perception, the clustering had a limited effect, but attributes such as hardness, melting, and creaminess were more related to the droplet-matrix interactions.

The mm-scale oil droplet inhomogeneity had limited effect on the mechanical properties but affected the sensory perception considerably. When the oil content of the dispersed phase is higher, most sensory attributes are affected; hardness, oiliness and oil flavour decreased, while mouth coating, lingering, and stickiness increased.

Overall, we showed that sensory properties of an emulsion-filled gel are a result of an interplay between oil droplet distribution and droplet-matrix interactions.

## Appendix A.

### A theoretical model for the elastic modulus

The mechanical properties of emulsion-filled gels can be described with different theoretical models. Classical approaches include the Kerner model (Kerner, 1956) for compressible composite gels and the Palierne model for incompressible materials (Palierne, 1990). These models do not include the effect of an inhomogeneous distribution. To include the effect of inhomogeneous droplet distribution, van Aken et al. (van Aken et al., 2015) proposed a modified version of the Kerner model. In this model, the region containing the clusters is seen as a distinct region with a different elastic modulus as the surrounding matrix (Oliver, Berndsen, van Aken, & Scholten, 2015a, b).

The modulus of the total gel, Egel, can then be expressed as:

$$\frac{E_{gel}}{E_{outer}} = \frac{15 * (1 - v_m)(M_{cluster} - 1) * \varphi_{cluster}}{(8 - 10 * v_m) * M_{cluster} + 7 - 5 * v_m - (8 - 10 * v_m) * (M_{cluster} - 1) * \varphi_{cluster}} + 1 \qquad A1$$

where $M_{cluster} = \frac{E_{inner}}{E_{outer}}$

In this model, E represents the modulus, $v_m$ is related to the Poisson ratio of the droplets, $\varphi$ the volume fraction, and $M$ the ratio between the moduli of the inner and the outer phase.

We assume that the particles are distributed over the clusters and the outer matrix, where $\varphi_{inner,}$ is the volume fraction of fillers within the clusters, and $\varphi_{outer}$ the volume fraction of non-clustered fillers in the continuous phase. χ is the ratio between fillers in the outer matrix, $\varphi_{outer}$ and the total filler volume fraction, $\varphi_{total}$ The volume fraction of the clusters can then be described by:

$$\varphi_{cluster} = \frac{\varphi_{total} * (\chi - 1)}{\varphi_{total} - \varphi_{inner}} \; and \; \chi = \frac{\varphi_{outer}}{\varphi_{total}} \qquad A2$$

These clusters have a modulus defined as:

$$\frac{E_{inner}}{E_{matrix}} = \frac{15 * (1 - v_m)(M_{inner} - 1) * \varphi_{inner}}{(8 - 10 * v_m) * M_{inner} + 7 - 5 * v_m - (8 - 10 * v_m) * (M_{inner} - 1) * \varphi_{inner}} + 1 \quad A3$$

where $M_{inner} = \frac{E_{filler}}{E_{matrix}}$ .

The modulus of the outer phase is consequently dependent on the amount of non-clustered fillers, $\chi * \varphi_{total}$, and is given as

$$\frac{E_{outer}}{E_{matrix}} = \frac{15 * (1 - v_m)(M_{outer} - 1) * \chi * \varphi_{total}}{(8 - 10 * v_m) * M_{outer} + 7 - 5 * v_m - (8 - 10 * v_m) * (M_{outer} - 1) * \chi * \varphi_{total}} + 1 \quad A4$$

where $M_{outer} = \frac{E_{filler}}{E_{matrix}}$ . It is pointed out here that $M_{inner}$ and $M_{outer}$ are not the same but differ in the filler they refer to.

The stiffness of the individual oil droplets can be estimated considering the interfacial tension, $\gamma_{ow}$ , and the size of the oil droplets as $E_{filler} = \frac{6 * \gamma_{ow}}{r}$ . Taking a surface tension ($\gamma_{ow}$) for gelatine-stabilized oil-water interfaces as 60 mN/m (Fainerman, Miller, & Joos, 1994), and a droplet radius of 1.5 μm, we obtain an estimated droplet stiffness of 240 kPa for the sunflower oil droplets. For DATEM-stabilized oil droplets with a surface tension of 10 mN/m (Krog, 1991), a stiffness of 30 kPa is found. For WPI stabilised droplets, we assume a stiffness of 103 kPa, based on literature (Oliver, Scholten, & van Aken, 2015a, b).

# Chapter 5 - Influence of clustering of protein-stabilised oil droplets with proanthocyanidins on mechanical, tribological and sensory properties of o/w emulsions and emulsion-filled gels

**5**
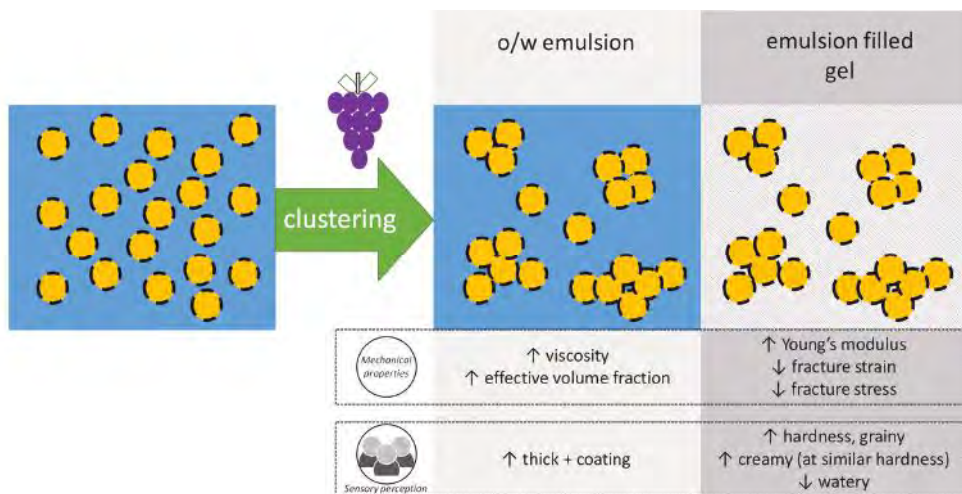
## Abstract

This study aimed to determine the effect of clustering of protein-stabilised oil droplets with proanthocyanidins on mechanical, tribological and sensory properties of o/w emulsions and emulsion-filled gels. Whey protein-stabilised oil droplets in o/w emulsions were crosslinked with proanthocyanidins, which led to the controlled formation of dense clusters of strongly-interacting oil droplets, in a size range from 2 to 110 μm. With increasing degree of clustering of oil droplets, the viscosity of o/w emulsions increased by up to three orders of magnitude. Clustering of oil droplets decreased friction coefficients. Clustering led to an increase in perceived creaminess, coating and thickness intensity. The changes in fat-related sensory perception were an interplay of both flow- and friction behaviour. In emulsion-filled gelatine gels, crosslinking of oil droplets increased Young's modulus and decreased fracture strain and stress. With increasing cluster size, gels were perceived as harder and grainier than emulsion-filled gels with non-clustered oil droplets. Creaminess of emulsion-filled gels did not increase upon clustering, as hardness also increased. When Young's modulus and perceived hardness of the gels were matched, gels containing clustered oil droplets tended to be perceived creamier (not significant, p=0.07) and significantly less watery than gels with non-clustered oil droplets. We relate these effects to the role of the emulsion droplets as structuring agents and an increase of the effective volume fraction by clustering of oil droplets.

We conclude that clustering of protein-stabilised oil droplets with proanthocyanidins in o/w emulsions and emulsion-filled gels can be used to modify flow- and texture properties with positive effects on perception of fat-related sensory attributes.

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## 5.1 Introduction

Rheological, mechanical and tribological properties of o/w emulsions and emulsion-filled gels largely influence the sensory perception of these foods. For example, for liquid o/w emulsions, the viscosity has been related to perceived thickness (Cutler, Morris, & Taylor, 1983; Akhtar, Stenzel, Murray, & Dickinson, 2005; Scholten, 2017; van Aken, Vingerhoeds, & de Wijk, 2011). For emulsion-filled gels, fracture stress has been related to perceived firmness/hardness (Çakır et al., 2012; Young, Cheong, Hedderley, Morgenstern, & James, 2013; van Vliet, van Aken, de Jongh, & Hamer, 2009). For both emulsions and emulsion-filled gels, the properties of the dispersed oil phase (*e.g.* oil droplet size and number, volume fraction and interactions of oil droplets with the gel matrix) contribute to the rheological, mechanical and tribological properties and consequently to the sensory properties (Mao & McClements, 2012a, b; Mosca, Rocha, Sala, van de Velde, & Stieger, 2012; Sala, van Vliet, Cohen Stuart, Aken, & van de Velde, 2009; Foegeding, 2007). In o/w emulsions, decreasing oil droplet size has been shown to increase the perception of fat-related sensory attributes at constant oil volume fraction, such as creaminess and smoothness (Lett et al., 2016). Such an increase in creaminess perception can also be obtained by increasing the oil volume fraction (Laguna, Farrell, Bryant, Morina, & Sarkar, 2017).

Not only the quantity and size of oil droplets influence sensory properties of o/w emulsions and emulsion-filled gels, but also the spatial distribution of oil droplets in the foods. The spatial distribution of oil droplets can be homogeneous or heterogeneous, with microscopic or macroscopic oil-enriched and oil-depleted areas (Oliver, Berndsen, van Aken, & Scholten, 2015a, b; Oliver, Wieck, & Scholten, 2016). It has been shown that clustering of oil droplets changes the rheological and mechanical properties of liquid o/w emulsions (Fuhrmann, Sala, Stieger, & Scholten, 2019a; Mao & McClements, 2011) and emulsion-filled gels (Oliver et al., 2015b). In o/w emulsions, clustering of oil droplets increases viscosity, whereas, in emulsion-filled gels, clustering of oil droplets increases Young's modulus, provided that the oil droplets are bound to the gel matrix (Sala, Van Aken, Stuart, & Van De Velde, 2007). Both effects are caused by the entrapment of the continuous (aqueous) phase within the oil droplet clusters, thereby effectively increasing the dispersed oil volume fraction, which increases viscosity and Young's modulus (van Aken, Oliver, & Scholten, 2015). Another aspect that contributes to changes in rheological and mechanical properties is that oil droplet clusters are typically non-spherical but randomly-shaped. Non-spherical filler particles display larger hydrodynamic diameters than spherical objects, thus occupy more volume than spherical clusters. Also, when a non-spherical filler in a gel is compressed, the response in terms of stress is more complex and in general larger than when a spherical filler is compressed.

The degree of oil droplet clustering, oil droplet cluster size, and interactions within an oil droplet cluster (oil droplet cluster strength) strongly influence the rheological properties of o/w emulsions and the mechanical properties of emulsion-filled gels. Consequently, oil droplet clustering is a versatile tool to structure oil droplets in fat-containing foods to tune mechanical and sensory properties.

In our previous studies, we have shown that clustering of oil droplets in emulsions can increase creaminess and thickness perception (Fuhrmann et al.,2019b). Previously, the oil droplets were clustered by mixing o/w emulsions stabilised with oppositely-charged emulsifiers (Fuhrmann et al., 2019a). These electrostatic attractions in emulsions are relatively weak physical interactions, resulting in formation of oil droplet

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clusters that are weakly interacting and loosely clustered. It is known that the type of interactions between droplets alters the mechanical properties of the clusters by changing the packing density of the individual oil droplets. When oil droplets within clusters are interacting strongly, or the number of interactions among oil droplets increases, the clusters become denser and subsequently, less continuous phase is entrapped (Fuhrmann et al., 2019a). Strong interactions could decrease the deformability of the oil droplet clusters, and as such, have a different effect on the rheological or mechanical properties. In addition, strongly interacting clusters may also be more stable when incorporating them into emulsion-filled gels. Loose and weak clusters formed by hetero-aggregation displayed low shear stability and disintegrated upon shearing, making it difficult to incorporate them into emulsion-filled gels (Fuhrmann, Sala, Stieger, & Scholten, 2020a). Using denser and strongly interacting clusters may, therefore, be beneficial and may allow to incorporate the oil droplet clusters into emulsion-filled gels. Consequently, the objective of this study was to investigate the effect of clusters induced by strong interactions on the mechanical, tribological and sensory properties of emulsion-filled gels and o/w emulsions. We used proanthocyanidins as a cross-linking agent to strongly crosslink protein-stabilised oil droplets in o/w emulsions (Fuhrmann et al., 2019a). These clusters have indeed been shown to be dense and they are not spherically shaped but can be considered more randomly-shaped. The attractive interactions between proanthocyanidins and proteins are based on a high affinity through several types of interactions, including H-bridges, covalent bonds and molecule (π-π) stacking (Bohin et al., 2014; Bohin, Vincken, Van Der Hijden, & Gruppen, 2012; Ozdal, Capanoglu, & Altay, 2013). Proanthocyanidins are beneficial to control cluster size, as deactivation of enzymes (Matalanis & McClements, 2012) (*e.g.* transglutaminase) or chemical crosslinking is not required (Maier, Oechsle, & Weiss, 2015) (*e.g.* glutaraldehyde). We suggest that clustering of protein-stabilised oil droplets with proanthocyanidins provides a novel methodology allowing to control cluster size and strength, which can be applied in emulsion-filed gels. We hypothesise that creating emulsion droplet clusters with high cluster strength allows (a) to obtain clusters with high stability, permitting the incorporation of clusters in emulsion-filled gels, (b) to alter the mechanical properties of emulsion-filled gels and improve fat-related perception, (c) to obtain emulsions with improved fat-related perception. To better understand the changes in sensory perception during oral processing, we took the effect of the addition of saliva on rheological and tribological properties into account. The physical properties were correlated to the sensory properties to gain insights into which physical properties drive sensory perception of o/w emulsions with clustered oil droplets.

## 5.2 Materials and methods

### 5.2.1 Materials

Whey protein isolate (BiPRO, WPI) was bought from Davisco (Lot # JE 062-3-420, USA). Gelatine Type A, (250 PS 30 with an IEP of 8-9) was acquired from Rousselot (Lot #1207647, The Netherlands). Vitaflavan® (grape seed extract, LES DÉRIVÉS RÉSINIQUES ET TERPÉNIQUES, Dax, France) was used as a source of proanthocyanidins. The grape seed extract contained 78.4% proanthocyanidins, of which, according to product specifications, less than 25% were monomers. Anhydrous citric acid (p.a.), sodium hydroxide (p.a.), sodium phosphate dibasic (food grade) and Nile Red were acquired from Sigma Aldrich (St. Louis MO, USA). Demineralised water was used for all experiments (MiliQ® system, Merck Millipore, Germany). Sunflower oil (Reddy, The Netherlands), sweetener (aspartame and acesulfame-K, "Canderel", The

Netherlands) and raspberry aroma (Lorann Bakery Emulsion "Raspberry", USA) were purchased at a local retailer.

### 5.2.2 Sample preparation

#### Preparation of o/w emulsions with clustered oil droplets

Oil-in-water (o/w) stock emulsions of 40% (v/v) were prepared with whey protein isolate (WPI) as an emulsifier. WPI (7.5 mg/mL) was dissolved overnight in a 0.12 M citric acid-phosphate buffer at pH 3. After dissolving WPI, the pH of the aqueous phase was adjusted to pH 3 with HCl (0.1 M) when necessary. Sunflower oil, 40% (v/v), was added to the aqueous phase while pre-homogenizing the emulsion with a rotor-stator homogeniser (Ultra-Turrax T25, IKA, Germany) at 8000 rpm for 3 min to obtain a pre-emulsion. Subsequently, the pre-emulsion was homogenised in a 2-stage homogeniser (PandaPlus, Niro Soavi, Parma, Italy) at 50 and 250 bar for two cycles to obtain a stock o/w emulsion. This stock emulsion was diluted using a citric acid-phosphate buffer (pH 3) to a final oil volume fraction of 0.2, which was used to prepare o/w emulsions with clustered oil droplets. The stock emulsions (volume fraction of 0.4) were processed without further dilution to prepare emulsion-filled gels.

O/w emulsions were clustered by adding a stock solution (20 mg/mL) of proanthocyanidins (GSE) to the emulsion. 20 mg/mL GSE was dissolved in water and stirred for 1 h to prepare the GSE stock solution. The stock solution was subsequently mixed with the WPI-stabilised o/w emulsions in different amounts to change the ratio between GSE and proteins. 0.25 g, 0.5 g, and 0.75 g GSE per g emulsifying protein were added to the o/w emulsions to vary the degree of clustering, thus to change oil droplet cluster size. Dilution effects due to the addition of GSE stock solutions were considered negligible. Following the addition of GSE stock solutions, o/w emulsions were vigorously mixed and stored for 24 h at room temperature to allow clustering of oil droplets. The different ratios GSE/WPI lead to the formation of small, medium and large clusters of oil droplets. In the remainder of the paper, we use the ratio of cross-linker to WPI to code the o/w emulsions: GSE0 (non-clustered o/w emulsions), GSE25 (o/w emulsions with small oil droplet clusters (0.25 g GSE/g WPI)), GSE50 (o/w emulsions with medium-sized oil droplet clusters (0.50 g GSE/g WPI)), and GSE75 (o/w emulsions with large oil droplet clusters (0.75 g GSE/g WPI)). The total oil volume fraction of all emulsions was 0.20.

For samples used for sensory testing, 1.25% (w/w) sweetener and 2% (v/w) raspberry aroma were added to provide a more pleasant flavour to the o/w emulsions. All samples were prepared with food-safe ingredients and in a food-safe environment and were stored for a maximum of 5 days at 4°C. The term food-safe refers to conditions that fit the purpose for human consumption, not creating a food-safety hazard. An overview of the o/w emulsions can be found in Table 1.

**Table 1. Composition of o/w emulsions and emulsion-filled gels with clustered oil droplets. All emulsions and gels had an oil volume fraction of 0.20. The sample with * was included only for sensory testing. All emulsion-filled gels had a gelatine concentration of 5% in the aqueous phase. Significant differences (p<0.05) are indicated by different letters.**

| Sample | Code | GSE (g/g protein) | Size of oil droplets/Size of oil droplet clusters [D4,3] (µm) |
|---|---|---|---|
| **O/w emulsions** | | | |
| *Non-clustered o/w emulsion* | GSE0 | 0 | 3.6±0.2[d] |
| *Small clusters* | GSE25 | 0.25 | 16±2[c] |
| *Medium clusters* | GSE50 | 0.50 | 70±7[b] |
| *Large clusters* | GSE75 | 0.75 | 110±3[a] |
| **Emulsion-filled gels** | | | |
| *Non-clustered* | GSE0g | 0 | 6±1[d] |
| *Small clusters* | GSE25g | 0.25 | 18±4[c] |
| *Medium clusters* | GSE50g | 0.50 | 67±7[b] |
| *Non-clustered droplets, modulus matched (to GSE50g) \** | GSE0_gh | 0 | 6±1[d] |

Preparation of emulsion-filled gelatine gels with clustered oil droplets

Gelatine was dispersed in demineralised water at a concentration of 8% (w/v). The dispersion was heated in a water bath at 80˚C for 30 min. After the gelatine was dissolved, the solution was cooled to 30°C. While still liquid, the gelatine solution was mixed in a 50/50 ratio with o/w emulsions with clustered oil droplets (GSE25, GSE50 and GSE75) or o/w emulsions with non-clustered oil droplets (GSE0) at an oil volume fraction of 0.40 by gently stirring with a spoon. The final oil volume fraction in all emulsion-filled gels was 0.20, and the final gelatine concentration in the aqueous phase of all emulsion-filled gels was 5% (w/v). The mixture was transferred to a 60 mL syringe lubricated with paraffin oil and placed in an ice-bath to gel. The emulsion-filled gels were stored overnight at 4˚C. Before mechanical testing and sensory evaluation, emulsion-filled gels were equilibrated to room temperature for 2 h. Gels were coded according to the ratio of cross-linker GSE to WPI used to prepare the o/w emulsions, and the letter "g" was added to the sample code to indicate the gelled matrix: GSE0g (emulsion-filled gel with non-clustered oil droplets), GSE25g (emulsion-filled gel with small oil droplet clusters) and GSE50g (emulsion-filled gel with medium-sized oil droplet clusters). Emulsion-filled gel with large oil droplet clusters based on GSE75 o/w emulsions were not included, due to limitations in mixing to obtain a homogeneous cluster distribution. An overview of the emulsion-filled gels can be found in Table 1. For sensory testing, a control emulsion-filled gel with similar Young's modulus as the emulsion-filled gel with the larger oil droplet clusters (GSE50g) was prepared from an o/w emulsion with non-clustered oil droplets (GSE0), named GSE0_gh. The increased Young's modulus was obtained by increasing the final gelatine content in the continuous phase to 5.6% (w/v). We refer to this sample as GSE0_gh. All samples were prepared with food-safe ingredients and in a food-safe environment and were stored for a maximum of 5 days at 4 °C.

### 5.2.3 Physical characterisation of o/w emulsions and emulsion filled gels

#### Particle size

The droplet size distribution of the emulsions was determined using a static light scattering setup (Mastersizer, Malvern Mastersizer 2000S, Malvern Instruments Ltd., UK). The refractive index of the material was set at 1.45, and that of the dispersant at 1.33 (Khouryieh, Puli, Williams, & Aramouni, 2015). The particle diameter was reported as volume-weighted mean diameter D [4,3].

For emulsion-filled gels, the effective cluster size was estimated by microscopic imaging, as reported earlier for emulsions (Fuhrmann et al., 2019a). The pictures were taken as described in the next section on CLSM imaging. Subsequently, for 6 pictures per emulsion, the area of the present particles was determined using ImageJ/Fiji. From the particle area, an effective cluster diameter, referred to as cluster size, was calculated assuming a spherical shape of the clusters. The brightness was adjusted automatically. A threshold value was used to define a minimum particle size of 0.1 μm to remove background noise.

#### CLSM imaging

The oil phase of emulsion-filled gels was stained using 0.01% (w/v) Nile Red, added to the oil phase before homogenization. To facilitate the visualisation of oil droplet clusters, emulsion-filled gels with a reduced oil volume fraction (0.05) were made. CLSM images were recorded using a LEICA TCS SP5 Confocal Laser Scanning Microscope (Leica Microsystems CMS GmbH, Manheim, Germany) equipped with an inverted microscope (Leica DM IRBE). Samples were excited at 543 nm. The following filters were used: MBS: HFT 488/543 nm (Main Dichroic beam splitter) and DBS2 (secondary beam splitter) at 490 nm. The objective lens used was a Plan-Neofluar 10x/0.3 (Leica). Digital images were acquired at a resolution of 512x512 pixels with an image size of 1201x1201 μm. Image J/Fiji (1.51s) was used to adapt the contrast and brightness of the microscopic images obtained.

#### Rheological characterisation of o/w emulsions

Rheological properties were determined with an Anton Paar 302 Rheometer (MCR 302, Anton Paar GmbH, Graz, Austria) using a concentric cylinder cup geometry (sandblasted CC17S, probe diameter: 16.654 mm, cup inner diameter: 18.077 mm, according to producer). Gap size was set at 0.02 mm. Flow curves were determined by measuring viscosity at an increasing shear rate from 0.1-1000 s-1 in 30 min at 22°C. Measurements were performed in triplicates with newly prepared o/w emulsions. The averaged flow curves were fitted in the shear rate range 1-100 s-1 to the Ostwald-de Waele power-law model (Ostwald, 1925):

$$\eta = K * \dot{\gamma}^{n-1} \qquad\qquad 1$$

in which η represents viscosity (Pa·s), γ (s-1) shear rate, K flow consistency index (Pa·s$^n$) and n refers to the flow behaviour index, which indicates the magnitude of the shear-thinning behaviour (0 < n < 1).

#### Tribological characterisation of o/w emulsions

Tribological measurements of o/w emulsions were performed with an Anton Paar Rheometer (MCR 302, Anton Paar GmbH, Graz, Austria) equipped with a tribological setup (T-PTD 200). A glass ball-on-three polydimethylsiloxane-(PDMS)-pins setup (PDMS pins (r=2.8 mm), glass ball (r=6.3 mm)) was used. PDMS

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was chosen as the most suitable material to provide an alternative to oral surfaces and to represent some of the properties of oral surfaces. The properties of PDMS are currently considered to be closest to those of the tongue and palate, as discussed in detail by (Sarkar, Andablo-Reyes, Bryant, Dowson, & Neville, 2019).

600 µL of the o/w emulsion was loaded into the cell. A normal force of 1 N was applied. The rotational speed was increased and decreased in 4 cycles from 0.1 rpm to 1000 rpm (equivalent to 0.47 mm/s to 470 mm/s); run one and three performed an increasing speed from 0.1 rpm to 1000 rpm, while run two and four a decreasing speed from 1000 rpm to 0.1 rpm, in 300 s each. Torque and normal force were recorded, and friction coefficients, µ, calculated. The temperature was kept at 22°C with a temperature-controlled water bath. All measurements were performed in triplicates with new batches of o/w emulsions. Between each set of samples, pins were renewed to avoid wear of the tribological-pair. During the first run, we observed fluctuations in the measured friction coefficient due to adjustments of the PDMS pins to their final position and running-in effects. Results of the first run were therefore disregarded. The results of the third run (increasing speed) were used for further data analyses. From three individual measurements of the 3rd run, the friction coefficient, µ, was taken as a function of speed. Average friction coefficient in the boundary regime ($\mu_{bound}$), friction coefficients at 10 mm/s ($\mu_{10mm/s}$), 80 mm/s ($\mu_{80mm/s}$) and 200 mm/s ($\mu_{200mm/s}$) were extracted from the data. A power-law model,

$$\mu \sim speed^b \qquad\qquad 2$$

was used to fit the experimental data in the mixed lubrication regime, from which the exponent b was obtained from the best fit.

Uniaxial compression tests of emulsion-filled gels

Fracture stress, strain and Young's modulus of the emulsion-filled gels were determined with a Texture Analyser (TA.XT plus, Stable Micro Systems-SMS, equipped with a 50 kg load cell). Emulsion-filled gels, prepared in a syringe with a diameter of 23 mm, were cut into pieces with a height of 20 mm. Emulsion-filled gels were compressed with a disc-shaped aluminium probe with a radius of 50 mm. 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. A constant compression speed of 2 mm/s up to a compression strain of 80% was applied. Nine replicates (three pieces per specimen and three specimens per type of gel, each prepared separately) were measured, and the mean values for true fracture stress, true fracture strain and apparent Young's modulus were calculated following literature (Sala, van Vliet, Cohen Stuart, van de Velde, et al., 2009).

Tribological characterisation of emulsion-filled gels

Friction behaviour of emulsion-filled gels in contact with a PDMS probe was measured with a UMT Tribolab (Bruker, Billerica USA). As a lower drive, a reciprocating drive (5 mm stroke length, a frequency between 0 and 10 Hz) was used in combination with a liquid holder, lined with a smooth PDMS surface (60 mm x 40 mm, 4.5 mm thickness). The liquid holder was filled with the emulsion-filled gel, which was treated as previously described in literature (Liu, Stieger, van der Linden, & van de Velde, 2015). In short, emulsion-filled gels were extruded through the opening of a 60 mL syringe (BD Plastipak, Luer Lock) at a rate of 1

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mm/s, resulting in gel particles of roughly 1.5 mm in size. The broken-down emulsion-filled gel pieces were filled into a liquid holder to a height of 7 mm, and the upper probe was brought into contact with the gel pieces. A cylindrical upper probe (30 mm diameter, 15 mm height) with a rough surface and rounded edges were built in-house. The probe was made of PDMS, frequently used in food friction research (Dresselhuis, de Hoog, Cohen Stuart, & van Aken, 2008; Selway & Stokes, 2013). PDMS was mixed with the supplied crosslinker in a 10:1 volume ratio and subsequently de-aired for 2 h at room temperature. To provide roughness to the PDMS probe, the mould to prepare the cylindrical upper probe was coated with sandpaper (size 240, corresponding to an average particle diameter of 53 μm, according to ISO 6344 (1998)). The normal force was set to 0.5 N. The upper probe moved with increasing speeds from 0.01 Hz (equal to 0.1 mm/s) to 15 Hz (equal to 150 mm/s) in a linear movement relative to the lower probe. For each measurement, the extruded gel pieces completely covered the surfaces. The results obtained in time intervals during which a change of direction occurred were excluded from data analysis. All measurements were performed in triplicates with newly prepared samples. Saliva was not added as a lubricant during the tribological measurements, as we were specifically interested in the effects of clustering of oil droplets on the tribological properties of the gels.

### 5.2.4 Saliva addition to o/w emulsions

Human saliva was added to the o/w emulsions to mimic in-mouth conditions. Human saliva was collected as described by (Silletti, Vingerhoeds, Norde, & van Aken, 2007). A volunteer collected (unstimulated) saliva in cooled plastic tubes (Greiner centrifuge tubes 15 mL, Merck, US), after rinsing his mouth with water. The collection was performed mornings, and the volunteer was asked to abstain from food intake 2 hours before collection. Saliva was centrifuged at 10000 g for 30 min at cooled conditions to remove debris (Beckmann, Avanti TM J-25 I, JA-21, Beckman Coulter B.V. Mijdrecht, The Netherlands). The supernatant was collected and used within 4 h. Emulsions and saliva were combined in a 1:1 ratio and carefully mixed. To characterise the effect of saliva addition on the physical properties of o/w emulsions, oil droplet size, cluster size, rheological and tribological properties were determined as described in section 5.2.3. It was not within the scope of this research to investigate the effect of interpersonal differences of saliva on tribology, but rather a proof of principle.

### 5.2.5 Sensory evaluation

#### Sensory evaluation of o/w emulsions

Four o/w emulsions (GSE0, GSE25, GSE50 and GSE75) were evaluated by naïve participants using a "Rate-All-That-Apply" methodology (RATA) (Meyners, Jaeger, & Ares, 2015). The sensory evaluation was conducted with n=74 untrained participants (40 female, 34 males; mean age 22.8±3.8 years) recruited from the Wageningen University & Research campus. Participants were asked to give written informed consent before the study, confirmed the absence of any intolerance towards the ingredients present in the o/w emulsions and received financial compensation upon completion of the study. The research of this study does not fall within the remit of the 'Medical Research Involving Human Subjects Act'. The study was conducted in agreement with the ethical principles regarding human experimentation outlined in the Declaration of Helsinki. Participants received a digital copy of the sensory terms and explanations before participation (Table 2). O/w emulsions were presented in one session of 75 minutes in randomised order.

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20 mL of each o/w emulsion was served in 100 mL paper cups labelled with random 3-digit codes. Participants performed the RATA test with the o/w emulsions on a set of 13 attributes including texture, taste and after-feel attributes. Participants were instructed to take a sip of the o/w emulsion and swallow, wait for 20 s and then fill in the RATA questionnaire. The 20s waiting period was chosen based on a pre-test, as the attribute astringency typically required more time to be perceived. The time was indicated using a stop-watch integrated into the digital questionnaire. Between tasting of two samples, participants had a 2 min break and were asked to rinse their mouth with water and offered to eat white bread. Intensity rating was done using a 9-point scale with anchors "weak" and "strong" and the possibility to choose "not applicable". Data was collected using tablets with a questionnaire made in EyeQuestion (Version 4.11.3).

**Table 2. Sensory terms and descriptors for RATA of liquid o/w emulsions (1) and ranking tests of semi-solid emulsion-filled gels (2). Terms and descriptions were taken from Benjamins, Vingerhoeds, Zoet, de Hoog, & van Aken, 2009; Oppermann, de Graaf, Scholten, Stieger, & Piqueras-Fiszman, 2017; van Aken, Vingerhoeds, & de Wijk, 2011, and adjusted or amended where required.**

| Attribute | Definition |
|---|---|
| Astringency (1) | The intensity of astringent or rough feeling in the mouth. A chalky, rough feeling in the mouth (especially on the teeth). |
| Coating (1, 2) | The intensity of the perception of a coating feeling. The product perception remains after swallowing. The whole mouth is covered |
| Creaminess (1, 2) | The intensity of creaminess/softness. Creamy is a soft, full feeling in the mouth. It leaves a soft, fatty feeling. It is perceived in the whole mouth and gives a velvety feeling. Flows through the mouth; |
| Flavour intensity (1) | The intensity of overall flavour, *e.g.* raspberry aroma. |
| Graininess (1, 2) | This attribute can be judged by rubbing the tongue against the palate. An example of a grainy product is semolina pudding |
| Hardness (2) | The force needed to deform a sample. A sample requiring a lot of force (*e.g.* old cheese) is hard. Hardness can be assessed by using the tongue. |
| Homogeneity (1, 2) | A product that consists of one compound, it feels even. Custard is a homogeneous product. Yoghurt with granola is a not homogeneous product. |
| Melting (1, 2) | Samples which melt during consumption (*e.g.* chocolate, ice) Examples of samples that do not melt are crackers or bread. |
| Oiliness (1) | The intensity of an oily feeling in the mouth. It gives a smooth feeling and a coating on the palate. An oily layer that stays in the mouth |
| Sourness (1) | The intensity of sourness |
| Stickiness (1) | The intensity of stickiness. A sticky feeling that can be perceived with tongue and palate. An example of a sticky product is a caramel candy bar. It is perceived between the teeth during a chewing movement. |
| Sweetness (1) | The intensity of sweet flavour. Sweet taste |
| Thickness (1) | The intensity of thickness of the product in the mouth after taking a bite/sip. This attribute is perceived by moving the tongue up and down against the palate. If a product is very thin, it immediately spreads throughout the mouth. |
| Wateriness (2) | Thin, weak texture, opposite of viscous, lacking body. |

Sensory evaluation of emulsion-filled gels

Four emulsion-filled gels (GSE0g, GSE25g, GSE50g, GSE0_gh) were evaluated by untrained participants (n=50, 38 females, 12 males; mean age 25.8±2.2 years) who were recruited from the Wageningen University & Research campus. The samples were evaluated using a ranking approach, as differences in sensory perception between the emulsion-filled gels were expected to be smaller than the differences between the o/w emulsions. Participants were asked to sign informed consent, to confirm the absence of any intolerance towards the ingredients present in the emulsion-filled gels and received financial compensation upon completion of the study. Like the sensory evaluation of the o/w emulsions, the research of this study does not fall within the remit of the 'Medical Research Involving Human Subjects Act'. The study was conducted in agreement with the ethical principles regarding human experimentation outlined in the Declaration of Helsinki. Participants received a digital copy of the sensory terms used for the ranking tests and explanations before participation (Table 2). Emulsion-filled gels were presented

simultaneously in one session of 60 min in randomised order. 50 g of each emulsion-filled gel was served in 100 mL paper cups labelled with random 3-digit codes. Participants ranked four emulsion-filled gels (GSE0g_, GSE25g, GSE50g and GSE0_gh) on seven attributes (Table 2) with no ties allowed. The attributes for the ranking test were selected based on the outcomes of a preliminary RATA test of the emulsion-filled gels (results not shown). Participants were instructed to take a part of the emulsion-filled gel with a plastic spoon, process it freely in their mouth and swallow. Re-tasting of samples was allowed. Data was collected using a paper-based questionnaire made in EyeQuestion (Version 4.11.3).

### 5.2.6 Statistical data analysis

Statistical analysis of the RATA data for the o/w emulsions was done following the procedure described by Meyners (Meyners et al., 2016) and Oppermann (Oppermann et al., 2017). RATA data were considered as continuous intensity scores with "not applicable" being evaluated as 0 (Meyners et al., 2016). A two-way ANOVA was carried out. Significance levels were described using Tukey's Honest Significant Difference Test (HSD) at 95% confidence level. For the correlation analysis, partial least squares regression (PLSR) was performed. For the data obtained from the ranking test of the emulsion-filled gels, Friedman tests with an LSD post hoc test ($p < 0.05$) were performed to determine significant differences between ranks. R Studio (Version 1.0.143) was used with the additional packages SensoMineR, FactoMineR and pls.

## 5.3 Results and discussion

### 5.3.1 Physical properties of o/w emulsions with clustered oil droplets

Oil droplet cluster size, flow and tribological properties of the o/w emulsions are summarised in Table 3. The properties of o/w emulsions with added saliva (1:1) (Table 3) will be discussed in section 5.3.2.

**Table 3. Oil droplet size, flow and tribological properties of o/w emulsions with clustered oil droplets. The properties of the o/w emulsions are shown in the upper table, and those of the o/w emulsions after addition of saliva (1:1) are shown in the lower table. Significant differences (p<0.05) are indicated by different letters.**

| Sample | Code | Size | Consistency | Flow index | $\mu_{bound}$ | $\mu_{10mm/s}$ | $\mu_{80\,mm/s}$ | $\mu_{200\,mm/s}$ | b |
|---|---|---|---|---|---|---|---|---|---|
| | | [D4,3] | K | n | | | | | |
| | | (μm) | mPa s$^n$ | (-) | (-) | (-) | (-) | (-) | (/mm/s) |
| *O/w emulsions without saliva* | | | | | | | | | |
| **Non-clustered, oil droplets** | GSE0 | 3.6±0.2[g] | 3 | 0.99 | 0.11±0.03[de] | 0.13±0.01[cd] | 0.17±0.01[cd] | 0.08±0.01[c] | -0.36 |
| **Small clustered, oil droplets** | GSE25 | 16±2[f] | 19 | 0.81 | 0.09±0.02[e] | 0.10±0.02[d] | 0.13±0.01[d] | 0.06±0.01[c] | -0.32 |
| **Medium sized, clustered oil droplets** | GSE50 | 70±7[c] | 889 | 0.07 | 0.22±0.02[c] | 0.20±0.02[c] | 0.22±0.01[c] | 0.06±0.01[c] | -0.46 |
| **Large sized, clustered oil droplets** | GSE75 | 110±3[a] | 4919 | 0.01 | 0.18±0.03[cd] | 0.20±0.01[c] | 0.12±0.01[d] | 0.03±0.01[d] | -0.19 |
| *O/w emulsions with added saliva (1:1)* | | | | | | | | | |
| **Non-clustered, oil droplets** | GSE0 | 41±2[e] | 32 | 0.40 | 0.39±0.03[b] | 0.42±0.02[ab] | 0.31±0.01[b] | 0.13±0.01[ab] | -0.13 |
| **Small clustered, oil droplets** | GSE25 | 50±1[d] | 59 | 0.39 | 0.52±0.04[a] | 0.50±0.02[a] | 0.33±0.02[b] | 0.15±0.01[a] | -0.25 |
| **Medium sized, clustered oil droplets** | GSE50 | 73±2[c] | 115 | 0.16 | 0.49±0.05[a] | 0.37±0.08[b] | 0.40±0.03[a] | 0.14±0.01[a] | -0.43 |
| **Large sized, clustered oil droplets** | GSE75 | 99±2[b] | 398 | 0.01 | 0.40±0.02[b] | 0.46±0.01[ab] | 0.31±0.04[b] | 0.11±0.01[b] | -0.20 |

With increasing concentration of proanthocyanidins from 0 to 0.75g proanthocyanidins per g protein, average cluster size (D4,3) of o/w emulsions increased from 3 μm for non-clustered o/w emulsions to 110

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μm for o/w emulsions with large oil droplet clusters. This increase in cluster size can be explained by the larger degree of crosslinking between droplets to create larger clusters. Compared to other clustering methods such as hetero-aggregation, GSE-based clustering leads to considerably larger clusters with sizes of 100 μm, whereas hetero-aggregation results in clusters of roughly 50 μm (Fuhrmann et al., 2019a). In addition, it has already been shown that these clusters are denser than weakly aggregated clusters (Fuhrmann et al., 2019a). With increasing cluster size, the viscosity increased, as expected, from 3 mPa s to roughly 5000 mPa s, due to an increase in effective oil volume fraction. Clustering of oil droplets led to a shear-thinning flow profile. As indicated by the decrease of the flow index, n, from roughly 1 to 0.01, the steepness of the flow curve increased with increasing cluster size. The shear-thinning behaviour of the clustered emulsions can be attributed to the increase in order in the system upon shear stress and potential cluster destabilisation at high shear rates.

Cluster size also influenced the lubrication properties of o/w emulsions. Substantial differences among emulsions can be observed in the slope of the Stribeck curve in the mixed regime (exponent b) and for friction coefficients measured at speeds in the boundary and the mixed regime. The slope of the mixed regime, b, of non-clustered emulsions was -0.36, and that of small clusters was -0.32. For emulsions with medium-sized clusters, the exponent increased to -0.46. When the oil droplet clusters were even larger, (GSE75), b dropped to -0.19. We suggest that medium-sized clusters might have facilitated the formation of an oil droplet layer on the surface of the PDMS geometry or might have helped lubrication by covering asperities on these surfaces. Large clusters, however, were maybe too large to enter the gap between the surfaces efficiently. Thus, the ability to form a continuous droplet layer might have been hindered. Linked to the cluster size, we observed that for both medium-sized (GSE50) and large-sized (GSE75) oil droplet clusters, the boundary friction coefficient was higher (0.2) compared to that of non-clustered emulsions (0.09). This increase in $\mu_{bound}$ might also be indicative of an initial difficulty for larger clusters to enter the gap. At higher speeds of 80 and 200 mm/s, the friction coefficient decreased for large clusters (GSE75) compared to that of non-clustered emulsions (GSE0). Thus, at high speeds, apparently, the speed is high enough to allow clusters to enter and separate the two surfaces efficiently. At higher speeds, emulsions with larger clusters seem to provide more lubrication that non-clustered emulsions. These results also show that similarly to what we observed for hetero-aggregated emulsions, clustering can lead to an increase in lubricity compared to non-clustered emulsions, especially for smaller cluster sizes (Fuhrmann et al., 2019a). However, as such clusters aggregated by GSE are stronger and larger than weak clusters, the friction coefficient of the emulsions can also increase again when the clusters become too large. This shows that both size and stability of clusters may influence lubrication behaviour of clusters emulsions. Overall, we observe that clustering of oil droplets in o/w emulsions, as expected, changed both flow properties and lubrication behaviour and that these properties depend on cluster size.

### 5.3.2 Effect of saliva on physical properties of o/w emulsions with clustered oil droplets

When saliva was added to the o/w emulsions, the structure and the physical properties of the o/w emulsions changed (Table 3). Saliva addition led to a substantial increase in cluster size in o/w emulsions with non-clustered oil droplets (GSE0) and with small oil droplet clusters (GSE25). Oil droplet size did not change for o/w emulsions with medium-sized (GSE50) and large clusters (GSE75). We suggest that the increase in cluster size is most likely an effect of interactions between salivary proteins and emulsifier

(WPI) (Sarkar, Goh, & Singh, 2009). Saliva is known to contain mucins, which are negatively charged (Silletti et al., 2007). O/w emulsions were stabilised with whey proteins, which are positively charged at a pH of 3. Flocculation of oil droplets can occur due to the electrostatic attraction between whey proteins and salivary proteins; the salivary proteins might act as a bridge between oil droplets. Non-clustered oil droplets and small clusters have a large surface area, which facilitates this interaction. Therefore, non-clustered oil droplets and small clusters might efficiently interact with the proteins from the saliva to form larger clusters due to flocculation. In the case of o/w emulsions with medium and large oil droplet clusters (GSE50 and GSE75), the total outer surface area of the droplets available for interactions with salivary proteins is lower; this would lead to a lower sensitivity for aggregation with mucins. Also, the WPI might be less accessible to salivary proteins, as it already forms bonds with proanthocyanidins, and therefore, additional electrostatic interactions between the clusters and salivary proteins might be limited. The increase in cluster size due to flocculation was also reflected in changes in the rheological properties of the emulsions. The viscosity of o/w emulsions with no clusters or small clusters increased upon addition of saliva. The consistency, $K$, increased and the flow index, $n$, decreased. For o/w emulsions with medium-sized or large clusters, the consistency, $K$, decreased because of the dilution of the emulsion, confirming that no additional aggregation took place.

The addition of saliva to the emulsions increased the friction coefficients in both boundary and mixed regimes to values up to 0.5. We suggest that this is related to several phenomena. Firstly, the aggregation of the oil droplets might change the friction coefficient. However, based on the results of the emulsions without saliva, this does not explain an increase to a friction coefficient of 0.5. The difference is most likely also linked to the presence of the salivary proteins. In o/w emulsions with added grape seed extract (GSE), the salivary proteins can interact with non-bound proanthocyanidins not included in the clusters. Unbound proanthocyanidins could interact with salivary proteins leading to the formation of protein-aggregates (Charlton et al., 2002), which might increase friction. Furthermore, saliva might attach to the PDMS or glass surfaces (Bongaerts, Rossetti, & Stokes, 2007), thus change the surface properties of the PDSM towards a less hydrophobic state. Consequently, the oil droplets would be less wetting and friction between the glass and PDMS could increase. These results show that saliva addition strongly modifies cluster size, rheological and tribological properties of o/w and clustered o/w emulsions.

### 5.3.3 Sensory perception of o/w emulsions with clustered oil droplets

The mean intensity scores obtained for the selected sensory attributes with the RATA test of all o/w emulsions are shown in Table 4.

**Table 4. RATA mean intensity scores ($n_{participants}$=74) of all attributes together with a standard error of means for o/w emulsions with clustered oil droplets. Letters indicate significant differences between samples (p<0.05), F values are given per attribute.**

| Attribute | | | Homogeneous emulsion | Clustered emulsions | | |
|---|---|---|---|---|---|---|
| | | | GSE0 | GSE25 | GSE50 | GSE75 |
| Astringent | F=10.5 | p<0.001 | 3.7 ± 0.3[c] | 4.1 ± 0.3[bc] | 4.7 ± 0.3[ab] | 5.4 ± 0.3[a] |
| Coating | F=2.6 | p<0.1 | 4.2 ± 0.2[b] | 4.5 ± 0.3[ab] | 4.5 ± 0.2[ab] | 4.9 ± 0.3[a] |
| Creaminess | F=8.8 | p<0.001 | 3.3 ± 0.2[b] | 2.7 ± 0.2[b] | 2.8 ± 0.2[b] | 3.9 ± 0.3[a] |
| Flavour | F=1.04 | p>0.1 | 6.1 ± 0.2[a] | 5.9 ± 0.2[a] | 5.7 ± 0.2[a] | 6.0 ± 0.2[a] |
| Graininess | F=23.7 | p<0.001 | 0.5 ± 0.1[b] | 1.1 ± 0.2[b] | 2.8 ± 0.3[a] | 2.1 ± 0.3[a] |
| Homogeneity | F=23.8 | p<0.001 | 7.2 ± 0.2[a] | 6.4 ± 0.3[b] | 5.2 ± 0.3[c] | 4.6 ± 0.3[c] |
| Melting | F=14.0 | p<0.001 | 1.3 ± 0.2[bc] | 1.0 ± 0.1[c] | 1.8 ± 0.3[b] | 2.3 ± 0.2[a] |
| Oiliness | F=5.4 | p<0.01 | 2.2 ± 0.3[b] | 2.8 ± 0.2[a] | 3.0 ± 0.2[a] | 2.2 ± 0.3[b] |
| Sourness | F=1.9 | p>0.1 | 5.9 ± 0.3[a] | 6.0 ± 0.2[a] | 6.2 ± 0.2[a] | 6.5 ± 0.2[a] |
| Stickiness | F=10.3 | p<0.001 | 1.8 ± 0.2[b] | 1.9 ± 0.2[b] | 2.5 ± 0.2[a] | 2.9 ± 0.3[a] |
| Sweetness | F=6.7 | p<0.001 | 4.1 ± 0.2[ab] | 4.1 ± 0.3[a] | 3.6 ± 0.2[bc] | 3.3 ± 0.2[c] |
| Thickness | F=42.5 | p<0.001 | 2.2 ± 0.2[c] | 2.4 ± 0.2[c] | 3.3 ± 0.2[b] | 4.8 ± 0.2[a] |
| Wateriness | F=22.3 | p<0.001 | 5.9 ± 0.3[a] | 5.6 ± 0.3[a] | 4.2 ± 0.3[b] | 3.6 ± 0.3[b] |

Emulsions with clusters were perceived significantly thicker than non-clustered emulsions (GSE0) with the same oil volume fraction. The increase in thickness is in line with the increase in viscosity of o/w emulsions upon clustering. Together with an increase in thickness and viscosity, wateriness perception decreased significantly from 5.9 for non-clustered emulsion (GSE0) to 3.6 for emulsions with large clusters (GSE75). Coating was also perceived with slightly higher intensities, as this was rated as 4.2 for non-clustered emulsions (GSE0) and increased to 4.9 for clustered emulsions with the largest clusters (GSE75). In the case of creaminess, only the emulsion with large clusters (GSE75), scored higher (3.9) than the non-clustered emulsion (3.3), which is also in line with the substantial increase in viscosity (Table 3). However, in the case of small and medium oil droplet clusters (GSE25 and GSE50), creaminess decreased to values of 2.7 compared to the non-clustered sample (GSE0). This finding suggests that creaminess was negatively affected by the clustering of oil droplets with strong interactions. The negative effect of clustering seems to be compensated by the positive effect of viscosity on creaminess for large clusters.

O/w emulsions with medium-sized oil droplet clusters (GSE50) and o/w emulsions with large oil droplet clusters (GSE75) were perceived as significantly more grainy than non-clustered o/w emulsions (GSE0). Graininess is most likely a consequence of the dense cluster structure and the high interaction strength within the oil droplet clusters due to cross-linking with proanthocyanidins. Clusters might have stayed intact during oral processing, leading to the perception of oil droplet clusters as particles. In the case of hetero-aggregated emulsions with weak electrostatic stabilisation, no increase in graininess was observed

(Fuhrmann, Kalisvaart, Sala, Scholten, & Stieger, 2019b). This suggests that the disintegration of the clusters or their deformability are important factors with regards to positive sensory evaluation. The deformability of these clusters may be lower than that of weak clusters, since the dense structure, because of strong interactions, most likely increases the cluster stiffness. This may then further contribute to graininess. This observation might also explain that creaminess was negatively affected by clustering, as graininess might have dominated creaminess. Concerning flavour perception, it is known that proanthocyanidins are perceived as astringent (Pascal et al., 2007). We indeed observed the occurrence of astringency in our study, and astringency increased with increasing cluster size, as higher concentrations of proanthocyanidins were used. The sensory data are summarised in a PCA biplot (Figure 1).
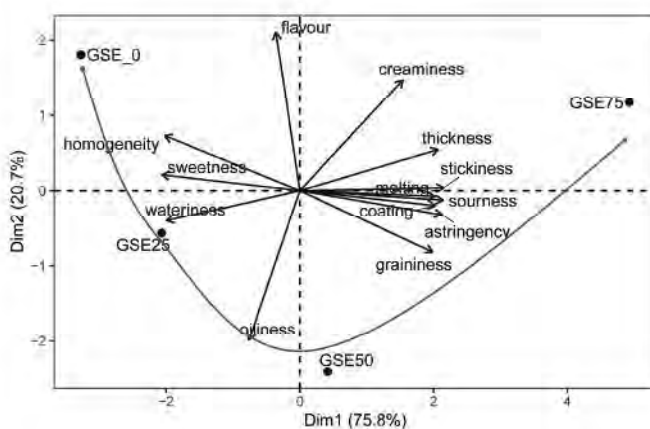


**Figure 1. PCA biplot of o/w emulsions with clustered oil droplets and sensory attributes determined using RATA ($n_{participants}$=74). The blue arrow is added to guide the reader on how cluster size increases throughout the PCA biplot.**

 The two first principal components explain about 97% of the variation. The four o/w emulsions are separated in the sensory space. The blue arrow in Figure 1 represents the pathway along which oil droplet cluster size increases. O/w emulsions are mainly separated in the first dimension (76%). Non-clustered o/w emulsions (GSE0), as well as emulsions with small clusters of oil droplets (GSE25), are mainly related to watery, sweet and homogeneous perception. It is interesting to note that o/w emulsions with medium-sized (GSE50) and large oil droplet clusters (GSE75) are separated in the PCA biplot, with o/w emulsions containing large clusters being mainly characterised by thick, creamy and sticky, while medium-sized clustered emulsions are related to thick, grainy, oily and astringent. While creaminess increased with an increase in cluster size for all clustered o/w emulsions, (from 2.7 to 3.9), oiliness was not directly linked to cluster size. For small and medium-sized clustered emulsions, oiliness was higher than for non-clustered emulsions and emulsions with large clusters. A possible reason could be a substantial increase in consistency (viscosity) when the cluster size is increased from medium to large. Consequently, perceived oiliness could have been reduced or masked. This phenomenon might also explain why graininess tends to be lower for large clusters compared to that of medium-sized ones. Additionally, the observed

difference in oiliness and creaminess between the emulsions could also occur from differences in lubricity between o/w emulsions. We have performed partial least squares regression (PLSR) to investigate further whether the physical properties correlate with sensory properties of o/w emulsions with clustered oil droplets (Figure 2).

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**Figure 2. PLSR plot of sensory attributes of o/w emulsions (with and without clustered oil droplets) (in red), emulsion properties (green) and emulsion properties after the addition of saliva ("+s"; blue).**
As expected, cluster size (D4,3), viscosity (K) and perceived thickness are highly correlated. Oil-related sensory attributes correlate more with lubrication properties. We have highlighted the results without saliva addition in green, and with saliva addition in blue. Creaminess seems to be inversely correlated to the friction coefficients in the boundary and mixed regime with saliva. This result is expected, as emulsions with better lubrication properties are typically found to be more creamy, due to their ability to form a lubricating film (Laguna et al., 2017). Oiliness shows a correlation to the parameter "b+s", which is the slope of the mixed regime, including the effect of saliva addition. The higher the absolute value of the parameter "b+s", the steeper the slope of this mixed regime and the faster the friction coefficient is reduced as a function of entrainment speed. This parameter may relate to the formation of a lubricating layer between the two entrained surfaces. We observe that o/w emulsions that were rated as oilier showed a considerably steeper slope, so a higher absolute "b+s" value. A similar relation between the slope of the mixed regime after addition of saliva and oily perception was also shown in previous work (Fuhrmann et al. 2019b), where clustering was induced by charge-based aggregation, and therefore the emulsions contained weaker clusters. Overall, we suggest that fat-sensory perception may be related to specific parameters of the lubrication behaviour, but more research is required to gain insight on how the perception and lubrication behaviour are linked.

### 5.3.4 Mechanical properties of emulsion-filled gels containing oil droplet clusters

Emulsion-filled gels with non-clustered oil droplets (GSE0g, Figure 3A) showed a homogeneous spatial distribution of the droplets, without clustering. In contrast, oil droplet clusters were observed, as expected, in emulsion-filled gels containing clusters of different sizes. As an example, a micrograph of a gel with medium-sized oil droplet clusters (GSE50g) is shown in Figure 3B. As can be seen, the clusters remained stable in the gel. In agreement with our earlier characterization (Fuhrmann et al., 2019a), the clusters were dense and randomly-shaped.
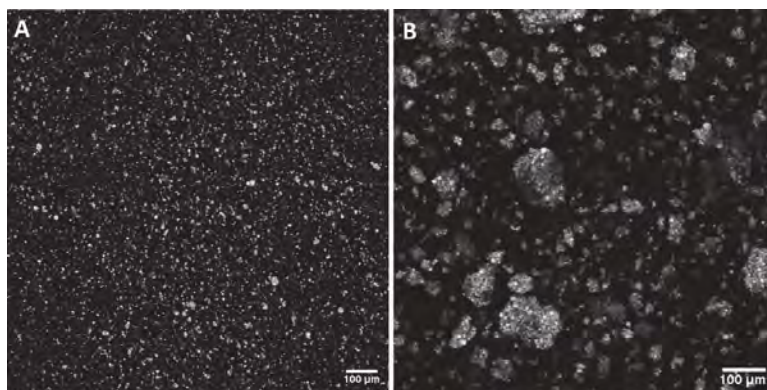


**Figure 3. CLSM micrographs of emulsion-filled gels with an oil volume fraction of 0.01. (A) non-clustered oil droplets (GSE0g) (red), (B) medium-sized clusters (GSE50g) (only oil phase is shown). Scale bar represents 100 μm.**
Incorporating the strongly-clustered o/w emulsions into a gelatine gel matrix changed Young's modulus, fracture stress and fracture strain (Table 5).

**Table 5. Young's modulus, true fracture strain and stress of emulsion-filled gels with non-clustered oil droplets, with non-clustered droplets and increased modulus, and with oil droplet clusters (small clusters, medium clusters). *relative to the matrix (gelatine gel without emulsion) Significant differences (p<0.05) are indicated by different letters.**

| Sample | Label | cluster size [μm] | Young's modulus (kPa) | relative modulus * $E_r$ [-] | True fracture strain [-] | True fracture stress [kPa] |
|---|---|---|---|---|---|---|
| Emulsion-filled gel with non-clustered, oil droplets | GSE0g | 6±1[c] | 12.8 ± 2.0[c] | 2.1 | 1.2 ± <0.1[a] | 25.0 ± 1.3[b] |
| Emulsion-filled gel with small, clustered oil droplets | GSE25g | 18±4[b] | 14.1 ± 2.6[bc] | 2.4 | 1.0 ± 0.1[b] | 15.7 ± 3.1[c] |
| Emulsion-filled gel with medium sized, clustered oil droplets | GSE50g | 67±7[a] | 17 ± 2.8[a] | 2.9 | 1.0 ± <0.1[b] | 17.6 ± 2.0[c] |
| Emulsion-filled gel with adjusted modulus (to GSE50g) and non-clustered, oil droplets | GSE0_gh | 6±1[c] | 16.1 ± 0.9[ab] | - | 1.2 ± <0.1[a] | 31.4 ± 1.1[a] |

Without oil droplets, Young's modulus of the unfilled gel was 10 kPa. Introducing non-clustered, oil droplets at a volume fraction of 0.20 into the gelatine matrix increased Young's modulus of the gel by 30% to 13 kPa. The incorporation of small oil droplet clusters into the gel matrix led to an additional increase of Young's modulus to 14 kPa. Upon increasing cluster size to 70 μm, Young's modulus increased to 17 kPa. This increase in Young's modulus with oil droplet clustering agrees with previous studies (Oliver et al., 2015b). Our findings underline that controlled clustering of oil droplets in emulsion-filled gels allows altering Young's modulus in a controlled manner.

True fracture strain slightly decreased with clustering of oil droplets, in agreement with literature (Sala, de Wijk, van de Velde, & van Aken, 2008), suggesting a decreasing fracture strain for an increasing effective oil volume fraction of bound droplets in a gel matrix. Fracture stress decreased by 40% upon clustering of oil droplets from 25 kPa (GSE0) to 17 kPa (GSE75). This reduction is in line with literature, showing decreasing fracture stress for polymer gels filled with an increasing volume fraction of bound oil droplets (Sala, van Vliet, Cohen Stuart, Aken, & van de Velde, 2009). Thus, we suggest that the observed changes in the fracture properties upon clustering of whey protein-stabilised oil droplets with proanthocyanidins in emulsion-filled gels, where the droplets are bound fillers, can be attributed to the increase in effective oil volume fraction.

### 5.3.5 Friction behaviour of emulsion-filled gels containing clustered oil droplets

To investigate the friction behaviour of emulsion-filled gels during mimicked oral processing, gels with clustered oil droplets (GSE0g, GSE25g, GSE50g and GSE0_gh) were extruded through a syringe, in a similar way as reported in literature (Liu et al., 2015), and the gel pieces were brought into contact with a PDMS surface as shown in Figure 4.
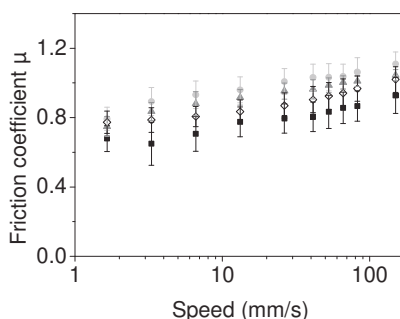


**Figure 4. Friction coefficient between emulsion filled gels (GSE0g, GSE25g, GSE50g and GSE0_gh) and a PDMS probe as a function of sliding speed (black squares: GSE0g, open diamond: GSE0_gh, grey triangle: GSE50g and grey circle: GSE25g). Measurements were performed in triplicates. Error bars represent standard deviation.**

Emulsion-filled gels with a homogeneous oil droplet distribution (GSE0g, black squares and GSE0_gh, open diamond) showed lower friction coefficients than emulsion-filled gels with clustered oil droplets (GSE25g, grey triangles and GSE50g, grey circles). This finding suggests that the clustered oil droplets may provide a certain roughness to the gel and change the gel-(particle) surface to such an extent that the friction forces increase. The reason for this could be found in the strong interaction between the oil droplets. Due to these strong interactions, clusters might behave like relatively hard non-spherical particles, which in consequence increases surface roughness, and thereby increase the friction between the gels and the probe. Such an increase in friction coefficient exerted by an increase in surface roughness has been described before for hydrogel materials synthesised on different substrates (Gong, 2006). Another contributing factor may be the hardness of the extruded gel-particles. Gels increased in hardness upon clustering of the oil droplets; thus, the harder particles might have increased friction.

### 5.3.6 Sensory properties of emulsion-filled gels with clustered oil droplets and correlations between sensory properties and physical measurements

The results of the ranking test of seven sensory attributes of the four emulsion-filled gels (GSE0g, GSE25g, GSE50g, GSE0_gh) are shown in Figure 5. To relate these sensory attributes to the physical properties of the emulsion-filled gels, we performed partial least squares regression (PLSR), shown in Figure 6.
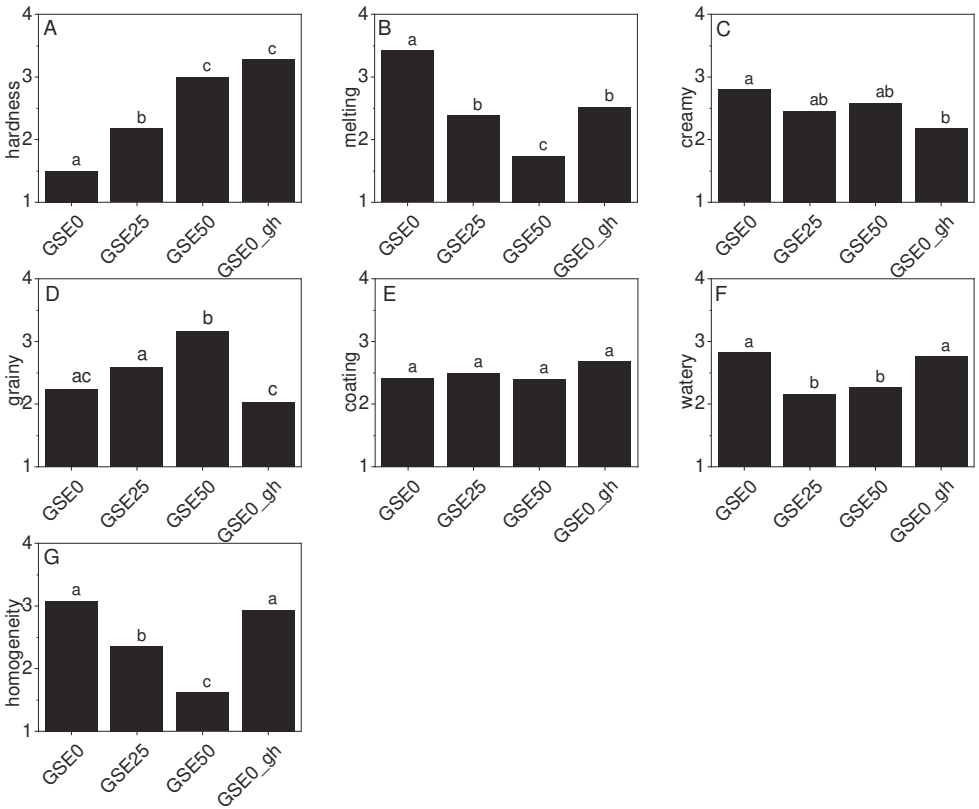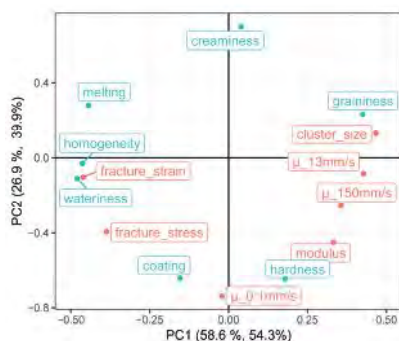


**Figure 5. Average ranks (n$_{participants}$=50) of emulsion-filled gels with single and with clustered oil droplets (GSE0g, GSE25g, GSE50g and GSE0_gh) for the attributes A) hardness B) melting C) creamy D) grainy E) coating F) watery and G) homogeneity. Different average ranks of emulsion-filled gels are indicated by different letters (p<0.05).**

**Figure 6. PLSR plot of sensory attributes of emulsion filled gels (with and without GSE) (in red), gel properties (blue). Principal components 1 and 2 of the sensory attributes explain 85% of the variance, while PC 1 and 2 of the gel properties explain 94% of the variance**

The sensory characteristics of the emulsion-filled gels changed significantly after clustering of the dispersed oil droplets. The attributes hardness, melting, grainy, watery, creamy and homogeneity were significantly affected by clustering of oil droplets. Gels with oil droplets included as clusters (GSE25g, GSE50g) were ranked as harder than gels without oil droplet clusters (GSE0g) (Figure 5A). This finding is in line with the increase in Young's modulus of the emulsion-filled gels with clustered oil droplets, compared to that of gels with non-clustered oil droplets (Table 5). Clustering of oil droplets thus seemed to increase the Young's modulus sufficiently to cause a perceivable increase in hardness. Correlations between Young's modulus and perceived hardness were reported previously (Wium, Qvist, & Gross, 1997).

Melting, watery and perceived homogeneity decreased upon clustering of oil droplets (GSE25g, GSE50g), compared to gels with non-clustered droplets (GSE0g) (Figure 5B, F, G). We suggest that interactions between gelatine and the proanthocyanidins could have contributed to the observed decrease in melting perception. Proanthocyanidins can act as a cross-linker for gelatine, thus decrease the thermo-reversible nature of the gels, as has been found earlier in literature (Liu et al., 2014). The decrease in perceived wateriness of the gels with increasing cluster size was probably due to the increase in effective oil volume fraction. As more of the continuous aqueous phase is entrapped within the clusters, the wateriness could decrease. A similar effect was found in liquid double emulsions (w/o/w), where replacement of oil by oil-entrapped water droplets was found to enhance fat-related sensory attributes (Oppermann et al., 2016).

The gels with clustered oil droplets were, furthermore, ranked as grainier than gels with non-clustered droplets (Figure 5D). This was also noticed in the emulsions. However, as detectability of particles is more difficult in solid-like foods than in liquid-like foods (Imai, Hatae, & Shimada, 1995), we expected that this effect would not be strong. Apparently, also in the emulsion-filled gels, the clusters could still be perceived. We propose several mechanisms contributing to this. Firstly, the clusters of the whey-protein stabilised oil droplets cross-linked with proanthocyanidins are very dense (Figure 3B). As previously discussed, such dense and probably irregularly-shaped clusters may be detected in the mouth and therefore were perceived as grainy. Previous studies on the incorporation of weaker clusters into emulsion-filled gels have

shown that less dense clusters are not detected (Fuhrmann et al., 2020a). This relation is confirmed in the PLSR (Figure 6), where cluster size and graininess are located near each other. Secondly, we observe a correlation (Figure 6) between graininess and the friction coefficients at lower speeds (13 mm/s). Clusters of oil droplets might increase the roughness of the gel surface, thus adding to the increase in graininess. Lastly, the changes in mechanical breakdown behaviour could contribute to graininess (Foegeding, 2007). Emulsion-filled gels with clustered oil droplets were more brittle than gels with non-clustered emulsion droplets (Table 5). This increase in brittleness could have contributed to an increased graininess, as the more brittle gels fall apart easier into smaller pieces during mastication; thus, the gels might have been perceived as grainier due to the presence of many stiff gel pieces. The results of the attribute homogeneity are in line with the changes in graininess.

Creaminess (Figure 5C) seems to decrease for the gels with oil droplet clusters, although the results were not significant. This slight decrease in creaminess is probably caused by an increase in the perceived sample hardness and the increase in graininess perception. A similar inverse relation between hardness and creaminess has been discussed in literature (González-Martín et al., 2011). To separate the effect of perceived hardness and droplet clustering on creaminess, we included an emulsion-filled gel (GSE_gh) with the same Young's modulus as the emulsion-filled gel with medium-sized clusters (GSE50g). The participants indeed ranked the samples GSE_gh and GSE50g as equally hard (Figure 5A). When comparing this emulsion-filled gel with non-clustered oil droplets (GSE0_gh) to the emulsion-filled gel with medium-sized oil droplet clusters (GSE50g) with the same overall gel hardness, we found that the sample with clustered droplets (GSE50g) tended to be ranked higher for creaminess (not significant, p=0.07) than the emulsion-filled gel with non-clustered oil droplets (GSE0_gh). This result suggests that the clustering of oil droplets itself possibly also contributes to an increase in perceived creaminess. Although creaminess was slightly affected by the clustering, coating perception did not vary significantly amongst gels (Figure 5E).

Overall, the results show that clustering of protein-stabilised oil droplets using proanthocyanidins changes the sensory perception of emulsion-filled gels considerably. With increasing cluster size, hardness and graininess increase, while melting and wateriness perception of emulsion-filled gelatine gels decrease. When comparing two emulsion-filled gels of similar perceived hardness and Young's modulus, clustering of oil droplets tended to increase creaminess (not significant, p=0.07). These changes in the sensory attributes are a combined effect of changes in mechanical properties, effective volume fraction, and interactions between oil droplets, cross-linker and the matrix. The clustering of the oil droplets may lead to a positive effect on creaminess. However, this seems to be counteracted by an increase in graininess, as the oil droplet clusters are sensed as particles during oral processing. These results indicate that the most positive effects on creaminess can be obtained when the clusters are large and strong enough to provide large effects on the rheological and mechanical properties, but they should also be weak enough to (partly) disintegrate during oral processing.

Based on these findings, we suggest that the controlled clustering of oil droplets with proanthocyanidins provides a useful approach to change the microstructure of emulsion-filled gels and manipulate their mechanical and sensorial properties.
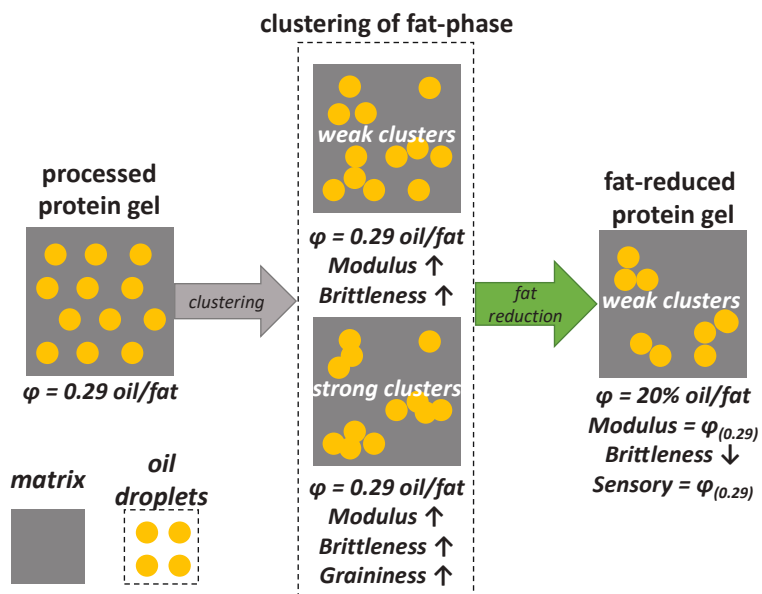
## 5.4 Conclusions

In this study, we aimed to determine the effect of proanthocyanidins-induced clustering of whey protein-stabilised oil droplets on mechanical, tribological and sensory properties of o/w emulsions and emulsion-filled gels. Clustering of oil droplets with strong inter-droplet interactions considerably influenced the physical and sensorial properties of both o/w emulsions and emulsion-filled gels. In emulsions, physical properties such as viscosity, lubrication behaviour, as well as fat-related sensory properties, including thickness and creaminess, increased upon oil droplet clustering, confirming our hypothesis. In emulsion-filled gels, we confirmed that the clustering of oil droplets increased the Young's modulus of the gels, while fracture strain and stress decreased. Because of the clustering of the oil droplets, gels were perceived as harder and less watery than gels with non-clustered droplets. Clustering of the oil droplets also shows a trend (non-significant) to increase the fat-related attributes, but only if accounted for the increase in Young's moduli due to the clustering. Although most changes in perception were positive, we also observed an increase in graininess. This was mainly due to the perception of large oil droplet clusters as particles. To prevent particle detection, clusters should, therefore, be strong enough to be incorporated in the products and change rheological properties, but also weak enough to fall apart during oral processing.

Overall, we found that the incorporation of clustered oil droplets is a useful and straightforward tool to structure emulsions and emulsion-filled gels. Controlled clustering with GSE allows creating stable emulsion droplet clusters in a variety of foods, which can be used to specifically target texture and fat-related sensory perception. Especially the cluster strength seems to be an important property for controlling the perception of thickness, wateriness, creaminess and coating. Cluster strength should not be too high to prevent graininess perception.

# Chapter 6 - The effect of fat-droplet clustering on the rheology and sensory perception of processed emulsion-filled particle gels

**clustering of fat-phase**

*weak clusters*

**processed protein gel**

*φ = 0.29 oil/fat*

*clustering*

*φ = 0.29 oil/fat*
*Modulus ↑*
*Brittleness ↑*

*fat reduction*

**fat-reduced protein gel**

*weak clusters*

*strong clusters*

*φ = 0.29 oil/fat*
*Modulus ↑*
*Brittleness ↑*
*Graininess ↑*

*φ = 20% oil/fat*
*Modulus = φ(0.29)*
*Brittleness ↓*
*Sensory = φ(0.29)*

*matrix*

**oil droplets**

## Abstract

Clustering of oil droplets has been shown to affect the mechanical and sensory properties of model emulsion-filled polymer gels. In this study, we investigated the effect of clustering of oil droplets on rheological and sensory properties of processed protein gels. Oil droplet clusters with weak (electrostatic) and strong (chemical crosslinking) attractive interactions were incorporated in gels to obtain a fat content of 20% (fat reduced) and 29% (full fat). To understand the effect of fat type on the investigated phenomena, the model foods were prepared with either anhydrous milk fat or sunflower oil. With increasing interaction strength of oil droplet clusters and solid fat content, the elastic modulus of gels increased up to 6-fold for solid milk fat ($p < 0.05$). Clustering of oil droplets decreased critical strain, indicating a more brittle emulsion-filled gel structure compared to emulsion-filled gels with non-clustered oil droplets. With increasing oil droplet cluster size, cross over stress, and critical strain decreased more. A fat-reduced emulsion-filled gel with oil droplet clusters stabilised with weak electrostatic interactions showed mechanical properties similar to those of a non-clustered full-fat emulsion-filled gel. Large, strongly interacting clusters led to the perception of brittleness and graininess, which are often undesired sensations for foods. Small, weakly interacting oil droplet clusters led to a slight reduction in perception of fat-related sensory attributes in sunflower oil-based gels. For milk fat-based gels, incorporation of small, weakly interacting oil droplet clusters did not significantly influence sensory properties. Fat-reduced processed protein gels with weakly interacting oil droplet clusters with 20% fat content had similar sensory properties as full-fat processed protein gels with non-clustered oil droplets with 29% fat content. We conclude that clustering of oil droplets in processed model gels modifies mechanical and sensory properties. The cluster interaction strength is a fundamental tuning parameter, as strong interactions between oil droplets in a cluster lead to dense clusters, which cause undesired graininess perception.

## 6.1 Introduction

Many food products are complex systems containing one or more dispersed phases in a continuous phase; typical examples are emulsion-filled gels, such as sausages or spreads. Mechanical properties of emulsion-filled gels depend on the physicochemical properties of the gel matrix and the emulsion oil droplets, as well as the interaction between oil droplets and matrix, oil volume fraction and oil droplet size (van Vliet, 1988).

With increasing oil volume fraction, the stiffness of emulsion-filled gels can increase or decrease depending on whether the oil droplets act as bound or unbound fillers (Sala, de Wijk, van de Velde, & van Aken, 2008). When the stiffness of bound dispersed emulsion droplets is higher than the matrix stiffness, the emulsion-filled gel is reinforced (Mor-Rosenberg, Shoemaker, & Rosenberg, 2004; Mor, Shoemaker, & Rosenberg, 1999). However, not only the stiffness of the droplets can change the gel modulus. Also, the heterogeneity of the spatial distribution of oil droplets can influence the stiffness of emulsion-filled gels (Fuhrmann, Sala, Stieger, & Scholten, 2020a; Oliver, Wieck, & Scholten, 2016; Fuhrmann, Sala, Stieger, & Scholten, 2019a). We have recently shown that small-scale clustering of oil droplets in emulsion-filled gels can be realised following two approaches: charge-based clustering of oil droplets via hetero-aggregation and clustering of protein-stabilized oil droplets by crosslinking with polyphenols (Fuhrmann, Sala, Stieger, & Scholten, 2020a; Fuhrmann, Sala, Stieger, & Scholten, 2020b). Both approaches allow changing oil droplet cluster size and interaction-strength among droplets within clusters. Protein-stabilised oil droplets crosslinked with polyphenols form strongly interacting, large and dense clusters. In comparison, oil droplets clustered by electrostatic attraction form weakly interacting, smaller and less dense clusters.

As a result of clustering of oil droplets, the continuous aqueous phase is entrapped between oil droplets and the effective volume of the dispersed phase increases (McClements, 2012). With an increase in effective oil volume fraction, a substantial change in mechanical and fracture properties of emulsion-filled gels has been observed (Fuhrmann, Sala, Stieger, & Scholten, 2020a; Oliver, Wieck, & Scholten, 2016). In emulsion-filled low acyl gellan gels, clustering of oil droplets led to an increase in Young's modulus with increasing cluster size, and a decrease in fracture stress and strain (Fuhrmann, Sala, Stieger, & Scholten, 2020a). Similar findings were described for emulsion-filled gelatine and carrageenan gels, in which an increasing oil volume fraction led to an increase in modulus and decrease in fracture stress and strain for bound fillers (Sala, van Vliet, Cohen Stuart, Aken, & van de Velde, 2009). Clustering of oil droplets also affects the sensory properties of emulsion-filled gels. Clustering was found to enhance texture attributes such as hardness and tended to increase fat-related sensory attributes such as creaminess (Fuhrmann, Kalisvaart, Sala, Scholten, & Stieger, 2019b). Thus, in model emulsion-filled polymer gels, clustering of oil droplets provides a methodology to tune mechanical and sensory properties.

It should be noted that studies on the effect of oil droplet clustering on mechanical and sensory properties of emulsion-filled gels used predominantly polymer gel matrices, like gelatine or gellan, rather than particle gels (e.g. whey protein gels or casein gels) (Fuhrmann et al., 2020a,b). Based on literature, it is known that oil droplets affect the rheological properties of particle gels differently than those of polymer gels (Sala, van Vliet, Cohen Stuart, van de Velde, & van Aken, 2009). It can be assumed that oil droplet clustering might affect particle gels differently than polymer gels.

To the best of our knowledge, the effect of clustering of oil droplets on mechanical and sensory properties has so far only been described for simple model emulsion-filled gels. Most foods undergo processing steps, such as shear and heat, during production, and contain many additional ingredients such as salts, preservatives, et cetera. Shear and heat may disintegrate oil droplet clusters preventing the incorporation of clusters in commercially available foods. It is also not known how clustering of oil droplets affects the structure, mechanical and sensory properties of particle gels, since studies so far investigated polymer emulsion-filled gels. We hypothesise that clustering of oil droplets in particle emulsion-filled gels can be used as a strategy to engineer the gel properties. This study aimed to investigate the effect of clustering of oil droplets on mechanical and sensory properties of complex protein-based particle gels.

## 6.2 Materials and methods

### 6.2.1 Materials
Whey protein isolate (WPI, BiPRO) was obtained from Davisco (Le Sueur, MN, USA). Gelatine (type A) was acquired from Rousselot (Son, The Netherlands). Sodium citrate dihydrate, sodium phosphate dibasic, sodium chloride, citric acid monohydrate and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Alexa Fluor 488-NHS was purchased at Interchim (France) and Bodipy 665/676 at Thermo Fisher Scientific SAS (France). Sodium hydroxide was purchased from Merck (MerckKGaA, Darmstadt, Germany). Quark (FrieslandCampina "magere kwark naturel") and sunflower oil were obtained from a local supermarket. Anhydrous milk fat (AFM) was kindly provided by FrieslandCampina (Wageningen, The Netherlands). Diacetyl tartaric acid ester of mono- and diglycerides (DATEM) was kindly provided by CP Kelco Inc (Atlanta, GA, USA). As a source of polyphenols, grape seed extract (Vitaflavan®) produced by Les Dérivés Résiniques et Terpéniques (Dax, France) was used. For all experiments, demineralised water was used (MilliQ® system, Merck Millipore, Germany).

### 6.2.2 Methods

#### 6.2.2.1 Preparation of o/w emulsions
An overview of all o/w emulsions and selected characteristics is provided in Table 1. We prepared processed model foods using milk fat, which is partly solid at room temperature, and sunflower oil. We did so to investigate whether fat hardness would affect the effect of clustering on gel properties.

*Preparation of o/w emulsions with non-clustered oil droplets*
To obtain o/w stock emulsions of 40% (v/v) fat, an aqueous phase containing 7.5 g/L WPI in water was prepared. Hydration of WPI was achieved by stirring the solution overnight at 4°C. The fat phase (liquid sunflower oil or melted anhydrous milk fat (80°C/30 min)) was added to the WPI solution at room temperature, and the mixture was pre-emulsified with a rotor-stator homogeniser (Ultra-Turrax, IKA, Germany) at 8000 rpm for 3 min. The pre-emulsions were homogenised for four cycles with a two-step, laboratory-scale homogeniser (PandaPlus, Niro Soavi, Parma, Italy) at 180 bar for the first stage and 30 bar for the second stage.

*Preparation of o/w emulsions with clustered oil droplets*

WPI-stabilised o/w emulsions cross-linked with polyphenols

O/w stock emulsions (40% (v/v) fat) with polyphenol-induced clusters were prepared with an aqueous phase containing 7.5 g/L WPI, dissolved overnight (4°C) in a 0.12 M McIlvaine buffer at pH 3. Emulsions were pre-emulsified and homogenised as described before. After homogenisation, 0.75 g GSE per gram of emulsifying protein was added as an aqueous stock solution of grape seed extract (GSE) of 200 g/L GSE in pH 3 McIlvaine buffer. Dilution due to the addition of this solution was considered negligible. After addition of GSE solution, emulsions were shaken and stored at room temperature for 24 h to allow for clustering of oil droplets.

O/w emulsions clustered by heteroaggregation

O/w emulsions (40% (v/v) fat) with hetero-aggregated clusters were prepared by combining a non-clustered DATEM-stabilised emulsion and a non-clustered gelatine-stabilised emulsion. O/w emulsions with gelatine were made by preparing an aqueous phase containing 9.6 g/L gelatine in a citric acid solution at pH 5 (set with 1 M NaOH). The gelatine dispersion was heated in a water bath at 80°C for 30 min. After the addition of the fat phase (40% (v/v)), the mixture was pre-emulsified and homogenised as described earlier. O/w emulsions with DATEM were prepared by dissolving DATEM in the fat phase to a concentration of 0.4 g/L and heating the solution under rotation at 80°C for 10 min. This fat phase (40% (v/v)) was added to an aqueous citric acid solution at pH 5 (set with 1 M NaOH) and prepared as described before. The non-clustered emulsions were stored at room temperature for 24 h. Hetero-aggregated emulsions were obtained by combining the two non-clustered emulsions at a volumetric ratio of 1:1. The mixture was shaken and stored for another 24 h before further use.

6.2.2.2 Formulation and production of processed emulsion-filled particle gels

Emulsion-filled particle gels with a fat content of 29% were produced using commercial quark as a protein-source and clustered- or non-clustered emulsions as a fat-source. Quark (85% moisture, <0.1% fat) was concentrated by centrifuging (Avanti J-26 XP with JLA-16250 rotor, Beckman Coulter, Fullerton, CA, USA) at 15000 rpm for 10 min. Centrifugation decreased moisture content of concentrated quark to 63% and increased protein content above 25%.

Concentrated quark, additional protein, salts and one of the previously described o/w emulsions were combined in defined ratios in a Thermomix (Thermomix TM31, Vorwerk Elektrowerke GmbH & Co. KG, Wuppertal, Germany).

The mixture was pre-sheared at room temperature. Subsequently, the blend was heated to 80°C. The product was then held at 80°C for a certain time, after which it was poured into containers. The product was stored for 2 days at 4°C before further analysis. The gels contained 29% or 20% fat.

For the sensory evaluation, an additional sample was produced with a reduced (milk-)fat content of 20% (w/w), using a gelatine-DATEM-clustered emulsion. This gel sample is abbreviated as "MF Gel-DAT lf". In total, 7 different samples were evaluated; 3 emulsion-filled gels prepared with liquid sunflower oil at 29% and 4 emulsion-filled gels with anhydrous milk fat as dispersed droplets, 3 of them with 29% milk fat and 1 with 20% milkfat. For the sensory evaluation, the samples were produced with food-grade ingredients

and in a food-safe environment. An overview of the samples and the used abbreviations can be found in Table 1.

**Table 1. Oil droplet/cluster sizes, rheological properties and abbreviations of o/w emulsions used to prepare processed emulsion-filled particle gels. WPI: whey protein isolate, GSE: grape seed extract (source of polyphenols), DATEM: diacetyl tartaric acid ester of mono- and diglycerides. MF: milk fat, SFO: sunflower oil, Gel: gelatine, non-C: non-clustered.**

| Emulsion | Milk fat | | | | | Sunflower oil | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Emulsion-filled gel* | *Abbreviation* | *Droplet/cluster size (µm)* | *Consistency K (mPa·sⁿ)* | *Flow index n (-)* | *Emulsion-filled gel* | *Abbreviation* | *Droplet/cluster size (µm)* | *Consistency K (mPa·sⁿ)* | *Flow index n (-)* |
| **Non-clustered** | Non-clustered, milk-fat | MF non-C | 1.0± 0.1[1] | 9 | 0.65 | Non-clustered, sunflower oil | SFO non-C | 2 ± 1[1] | 21 | 0.92 |
| **Hetero-aggregated** | Heteroaggregated (Gelatine-DATEM), milk-fat | MF Gel-DAT | 460 ± 12 | 4,695 | 0.19 | Heteroaggregated (Gelatine-DATEM), sunflower oil | SFO Gel-DAT | 38± 3[1] | 28,001 | 0.07 |
| **Cross-linked** | Cross-linked (GSE), milk-fat | MF GSE | 93 ± 30 | 28,000 | 0.21 | Cross-linked (GSE), sunflower oil | SFO GSE | 110 ± 3[2] | 49,192 | 0.01 |

[1]Fuhrmann et al., 2019a and [2]Fuhrmann et al.,2020a.

## 6.2.2.3 Microstructural analysis of processed emulsion-filled particle gels

*Determination of oil droplet size and oil droplet cluster size*

The droplet size distribution of non-clustered emulsions was determined by static light scattering using a Mastersizer 2000 (Malvern Instruments Co., Ltd., Worcestershire, UK). The refractive index of emulsion droplets was set at 1.45 and that of the dispersant at 1.33 (Khouryieh et al., 2015). Results are presented as Sauter mean diameter (d3,2).

To estimate oil droplet cluster sizes in the o/w emulsions, image analysis was used, as previously reported (Fuhrmann et al., 2019a). In short, emulsions were diluted with their respective aqueous phase and micrographs were taken with an optical light microscope (Axioskop 2 plus, Carl Zeiss AG, Germany) equipped with a camera (Axiocam ERc 5S, Carl Zeiss AG, Germany). Images were analysed, and particle size determined using ImageJ (Version) with a lower particle size threshold of 0.1 µm. The particle area was converted into a diameter assuming the spherical shape of clusters.

*Confocal scanning laser microscopy*

The microstructure of processed emulsion-filled particle gels was investigated using confocal scanning laser microscopy (CSLM). The fluorescent dyes Alexa Fluor® 488-NHS at 0.05% (w/v) and Bodipy® 665/676 at 0.05% (w/v) were dissolved together in dimethyl sulfoxide (DMSO). A thin layer (≈ 300 µm thick) of the sample (15 – 20 mg), was cut with minimal physical modification using a scalpel in order to keep the microstructure of the matrix as close as possible to that of the initial product. The sample is put on a microscopic flat glass slide topped with a 250 µm thick gene frame spacer (15x16mm, Thermo Fisher Scientific SAS, France). Three microliters of the fluorescent dyes solution are poured on the top of the sample and the dyes allowed to diffuse for 10 min in the sample. After the diffusion time, the excess of the dyes solution is removed using filter paper. The sample is covered with a cover-slip. The samples were taken using a Zeiss LSM 700 confocal microscope (Zeiss, France). For the image acquisition, the fluorescent dyes were excited at 488 nm and 639 nm respectively, and the fluorescence emission recorded at wavelength ranges of 400-565 nm and 600-700 nm, respectively. In the acquired images, fat is shown in red and protein in green. ImageJ/Fiji (1.51s) was used to adapt the contrast and brightness of the microscopic images obtained.

### 6.2.2.4 Physical characterisation of processed emulsion-filled particle gels

*Rheological characterisation*

The rheological properties of processed emulsion-filled particle gels were characterised using an Anton Paar MCR 302 rheometer (Anton Paar GmbH, Graz, Austria). For small-amplitude oscillatory strain (SAOS) measurements, a parallel-plate geometry with serrated stainless steel plate (diameter of 25 mm (PP25/P2-SS)) was used at a gap of 1 mm. To avoid drying out of the samples during measurements, the plate was covered on the edge with low viscous paraffin oil. Amplitude sweeps were performed at an angular frequency of 1 rad/s and a strain range of 0.01 to 100% at 20°C. We extracted elastic modulus G', loss modulus G'', cross-over strain (strain where G' and G'' crossed) and critical strain (10% deviation from the linear viscoelastic (LVE) range of G').

*Texture profile analysis*

Texture profile analysis (TPA) was performed using a texture analyser (TA XT-plus, Stable Micro Systems Ltd, Surrey, United Kingdom). The hot processed emulsion-filled particle gel-solution was poured into cylindrical moulds with a height of 40 mm and a surface area of 507 mm$^2$ and stored as described before analysis was performed (section 2.2.2). Processed emulsion-filled particle gels underwent a double compression to 25% of their original height at a speed of 1 mm/s, using a stainless steel cylindrical probe with a diameter of 75 mm. Hardness, cohesiveness, springiness, and adhesiveness were obtained from these data, as described in literature (Benjamin, Davidovich-Pinhas, Shpigelman, & Rytwo, 2018; García-Gómez, Vázquez-Odériz, Muñoz-Ferreiro, Romero-Rodríguez, & Vázquez, 2019). Young's modulus was calculated between strain rates of 0.01 and 0.025.

### 6.2.2.5 Sensory evaluation of the model processed emulsion-filled particle gels

Seven processed emulsion-filled particle gels (see Table 2) were evaluated by an untrained panel (n=83 participants; 60% female, 40% male; mean age 20.4 ± 5.0 years, age range from 19 to 60) using the Rate-All-That-Apply methodology (RATA) (Meyners, Jaeger, & Ares, 2016). Participants were mainly students, recruited from the Wageningen University & Research campus. Participants had to fulfil inclusion criteria, comprising good general health, no missing teeth (excluding wisdom teeth), no medication and no allergies/intolerances towards any of the ingredients. Participants gave informed written consent to take part in the study. Participants received financial compensation upon completion of the study. Before the participation, participants received a digital copy of the sensory attributes and their explanations. An overview of sensory attributes and explanations can be found in Table 3.

The processed emulsion-filled particle gels were presented in a randomised order in one session of 60 min. The sensory study was performed in meeting rooms with standard lighting and partitioning walls. 15 mL of each sample was served in a red 25 mL plastic cup labelled with a random 3-digit code. Participants rated the processed emulsion-filled particle gels on the provided attributes (Table 2). Subjects were instructed to take a portion of the sample with a spoon and keep the sample in their mouth for 10 seconds (timer). They were free to either spit out or swallow the sample. Participants were allowed to re-taste samples. Between samples, participants were instructed to drink warm water and eat crackers in order to remove the remaining sample. The RATA was done using a 9-point scale with anchors "weak" and "strong"

and the possibility to choose "not applicable". Data were collected online using tablets with a questionnaire made in EyeQuestion (Version 4.11.3).

**Table 2. Sensory attributes used to characterise the studied processed emulsion-filled particle gels and their descriptions.**

| Astringency | Mouth coating |
|---|---|
| The intensity of astringent or rough feeling in the mouth. A chalky, rough feeling in the mouth (especially on the teeth) like eating nuts or spinach. It can be perceived in the following places: at the front of the palate (border gum and teeth), at the back of the throat, at the back of the tongue, and the cheeks. | Degree of coating in the mouth. Residuals from food that stick to oral surfaces like the tongue and form a perceivable film. |
| **Cohesiveness** | **Oiliness** |
| The intensity of cohesiveness. The degree to which the sample deforms before rupturing when biting or applying force with the tongue. | The intensity of oily, fatty flavour. Taste of salad oil or fat. |
| **Creaminess** | **Saltiness** |
| The intensity of the creaminess/softness. This is a soft, full feeling in the mouth, which is also thick-soft and supple. The product is not rough and not dry. It leaves a soft, fatty feeling and is often in combination with fat. It is perceived in the whole mouth and gives a velvety feeling in the whole mouth. Flows through the mouth; velvety; warm; soft. | The intensity of a salty taste. Taste of kitchen salt. |
| **Graininess** | **Sourness** |
| The degree to which a product contains grains. This attribute can be judged by rubbing the tongue against the palate—air bubbles as granules as semolina pudding. | The intensity of a sour taste. Taste of lemons, pickles and yoghurt. |
| **Hardness** | **Stickiness** |
| The intensity of hardness. The force that the tongue or teeth apply to the product to compress the product. | The intensity of stickiness. This is a sticky feeling that can be perceived with tongue and palate. An example of a sticky product is the caramel of a Mars candy bar. It is perceived between the teeth during a chewing movement. The degree to which a product comes loose from different parts of the mouth determines the intensity of stickiness. |
| **Melting** | **Watery** |
| The degree to which a product becomes thin in the mouth and is spread throughout the mouth. | Amount of moisture perceived when tasting the product. |

### 6.2.2.6 Statistical data analysis

Statistical analysis of the RATA data was done as suggested previously (Meyners, Jaeger, & Ares, 2016). In short, RATA data were considered as continuous intensity scores with "not applicable" being evaluated as 0. A two-way ANOVA was carried out. Significance levels were described using Tukey's Honest Significant Difference Test (HSD) at 95% confidence level. Statistical data analysis was done using RStudio Version 1.1.423 (RStudio Inc.) and R 3.4.3, with the additional packages agricolae, dplyr and FactoMineR. The sensory data were, furthermore, visualised using a PCA. Significance levels for physical parameters were calculated based on ANOVA and Tukey's Honest Significant Difference Test (HSD) at 95% confidence level. The physical characterisation was performed in measurement and sample duplicates of two independent samples.

## 6.3 Results and discussion

### 6.3.1 Microstructure of processed emulsion-filled particle gels with and without oil droplet clusters

The size of oil droplet and oil droplet cluster in liquid emulsions and gels is reported in Table 1 in Materials and Methods. The microstructure of o/w emulsions before being introduced into the processed emulsion-filled particle gels is shown in Figure 1.
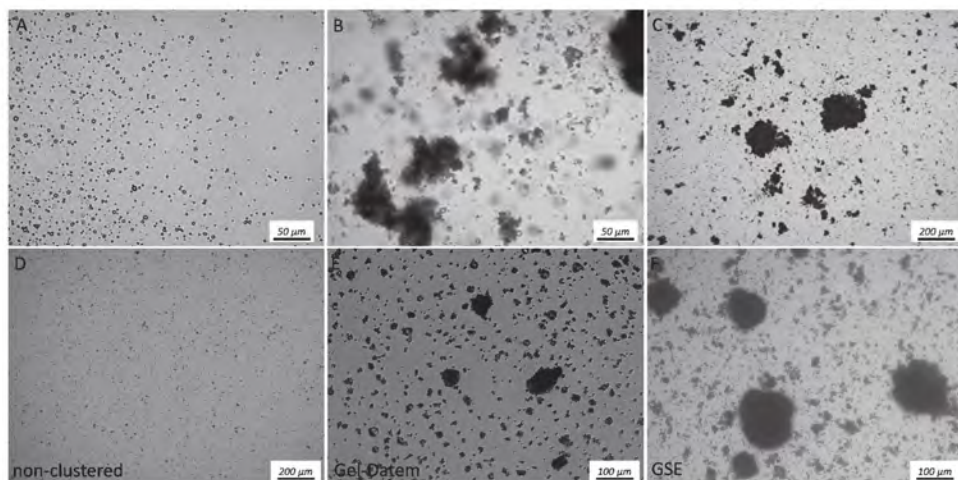
**Figure 1. Micrographs of o/w emulsions before incorporation into processed emulsion-filled particle gels.** Sunflower oil (A-C), milk fat (D-F). SFO non-C (A), SFO Gel-DAT (B), SFO GSE (C), MF non-C (D), MF Gel-DAT (E), MF GSE (F). Lines represent 50 μm (A-C), 200 μm (C,D) or 100 μm (E,F).

In both sunflower oil- and milk fat-based emulsions, droplets showed as expected a homogeneous distribution, and no aggregation in the "non-clustered" o/w emulsions was observed. For gelatine-DATEM-based hetero-aggregated o/w emulsions, droplet clustering was observed. When comparing the sunflower oil- and milk fat-based gelatine-DATEM emulsions, milk fat-based clustered emulsions seemed denser than sunflower oil-based ones (Figure 1), which could be attributed to the occurrence of partial coalescence (Boode, Walstra, & de Groot-Mostert, 1993). Partial coalescence of milk fat is a common phenomenon in dairy products. For both the oil-based and fat-based emulsions, the average size of the gelatine-DATEM clusters was comparable and around 40 μm. This result is in line with earlier studies (Fuhrmann et al., 2019a). For GSE-stabilised clusters, independently of whether liquid oil or solid fat was used as the dispersed phase, clusters showed a very dense structure, again in line with literature (Fuhrmann et al., 2019a). The average cluster sizes were found to be around 100 μm (Table 1).

Figure 2 provides representative CLSM micrographs highlighting the fat-phase (red) and protein phase (green) of processed emulsion-filled particle gels with and without oil droplet clusters.

**6**
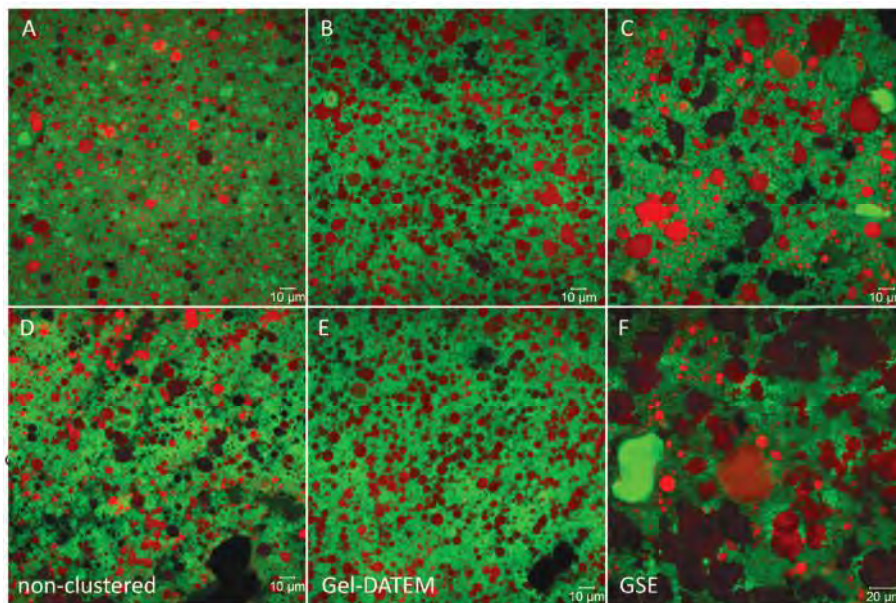


**Figure 2. CLSM micrographs of processed emulsion-filled particle gels (oil phase: red; protein phase: green). Sunflower oil (A-C), milk fat (D-F). Pictures A+D show the oil droplet distribution introducing a non-clustered emulsion; pictures B+E show the sample structure after introducing a Gelatine-DATEM hetero-aggregated emulsion; pictures C+F after introducing a GSE-clustered emulsion. Lines represent for A-E: 10 µm and for F: 20 µm.**

The pictures show that the processing steps, *i.e.* shear, heat and increase of the ionic strength compared to the emulsion, led to differences in the microstructure. Non-clustered (WPI-stabilised) sunflower oil droplets and milk-fat droplets remained relatively intact, and oil droplet coalescence was not observed. For hetero-aggregated clusters (Figure 2B/D), sunflower oil droplets clusters were slightly larger than in the liquid o/w emulsion, likely due to coalescence. For hetero-aggregated solid milk fat droplets, randomly shaped clustered structures were formed. For oil droplet clusters from liquid oil, we observed that shear, heat and the addition of melting salts reduced the cluster size, but did not lead to complete clusters disintegration. For protein-stabilised oil droplets clustered with polyphenols (GSE), we observe large clusters of fat. This suggests that a higher density of oil droplet clusters and stronger interactions between oil droplets within a cluster increase the stability of clusters against shear and heat treatment and allows to incorporate oil droplet clusters into processed gel-type foods.

## 6.3.2 Rheological properties of processed emulsion-filled particle gels with and without oil droplet clusters

Representative amplitude sweeps of the samples with milk fat are shown in Figure 3. A summary of all rheological parameters of the 7 samples is provided in Table 3.
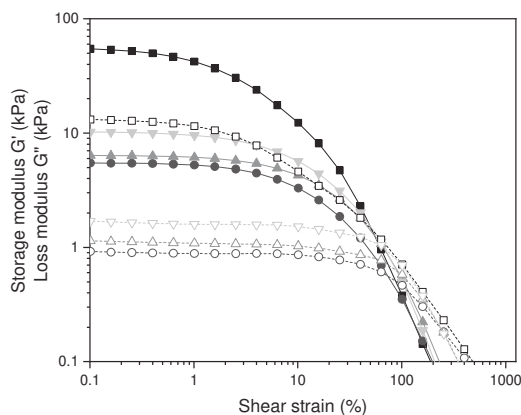
**Figure 3. Amplitude sweeps of processed emulsion-filled particle gels with milk fat emulsion. Storage modulus (G') is shown with filled symbols and loss modulus (G'') with open symbols. MF GSE (black square), MF Gel-DAT (light grey downward triangle), MF Gel-DAT (low fat) (mid-grey upward triangle), MF non-C (grey circle).**

**Table 3. Overview of rheological properties (± SD) of processed emulsion-filled particle gels obtained by small-amplitude sweeps. Different letters indicate a significant difference at p<0.05.**

| | Emulsion-filled gel | Code | G' in LVE (kPa) | G'' in LVE (kPa) | Young's Modulus (kPa at a strain of 0.01-0.025) | Cross over strain (%) | Critical strain G' (%) |
|---|---|---|---|---|---|---|---|
| *Fat-free matrix* | - | - | 1.1 ± 0.2e | 0.3 ± <0.1f | 4.2 ± 0.3d | 57.4 ± 2.6d | 2.84 ± 0.20c |
| Sunflower oil | | | | | | | |
| *Non-Clustered* | Non-clustered, sunflower oil | SFO non-C | 4.5 ± 0.4d | 0.7 ± <0.1e | 6.9 ± 1.11d | 82.7 ± 6.1b | 5.31 ± 0.34a |
| *Clustered* | Heteroaggregated (Gelatine-DATEM), sunflower oil | SFO Gel-DAT | 4.8 ± 0.5d | 0.8 ± <0.1de | 12.7 ± 1.5cd | 66.2 ± 2.6c | 4.30 ± 0.74b |
| | Cross-linked (GSE), sunflower oil | SFO GSE | 9.4 ± 0.6b | 2.2 ± 0.1b | 36.9 ± 1.0b | 54.7 ± 1.1d | 1.14 ± 0.17d |
| Milk fat | | | | | | | |
| *Non-Clustered* | Non-clustered, milk-fat | MF non-C | 5.5 ± 0.6cd | 0.9 ± <0.1de | 14.7 ± 2.7cd | 86.1 ± 4.4ab | 3.78 ± 0.13b |
| *Clustered* | Heteroaggregated (Gelatine-DATEM), milk-fat | MF Gel-DAT | 10.2 ± 0.1b | 1.7 ± <0.1c | 23.8 ± 4.3bc | 69.1 ± 0.2c | 2.68 ± 0.01c |
| | Cross-linked (GSE-WPI), milk-fat | MF GSE | 54.6 ± 0.8a | 13.1 ± 0.4a | 98.4 ± 15.4a | 49.7 ± 5.4d | 0.60 ± 0.04d |
| | Heteroaggregated (Gelatine-DATEM), milk-fat (20% fat) | MF Gel-DAT lf | 6.4 ± 0.2c | 1.1 ± <0.1d | 15.7 ± 0.2cd | 92.8 ± 2.0a | 4.10 ± 0.07b |

An increase of Young's modulus (1.64-fold) and elastic modulus G' (4-fold) was observed upon addition of sunflower oil to the matrix compared to the oil-free matrix, demonstrating that oil droplets were bound. The Young's modulus of processed emulsion-filled particle gels with solid milk-fat increased 3.5-times upon addition of fat to the matrix compared to the fat-free matrix. Similar increases were found for G'. The larger increase in Young's modulus is linked to the stiffness of the dispersed droplets, which were stiffer than the matrix, leading to a stronger reinforcement of the matrix which is in agreement with

previous studies (Sala, van Vliet, Cohen Stuart, Aken, & van de Velde, 2009). With increasing cluster size and cluster strength, the G' and Young's modulus of the processed emulsion-filled particle gels increased independently of the type of fat. Processed emulsion-filled particle gels with weakly interacting sunflower oil droplet clusters (SFO Gel-DAT) displayed a Young's modulus 1.5-times larger than non-clustered emulsion-filled gels (SFO non-C), and gels with strongly interacting sunflower oil droplet clusters (SFO GSE) displayed a Young's modulus 6-times larger compared to the non-clustered control (SFO non-C). Processed emulsion-filled particle gels with milk fat droplet clusters showed Young's moduli that were 1.6-times (MF Gel-DAT) and 6.7-times (MF GSE) higher compared to non-clustered processed emulsion-filled particle gels (MF non-C). Again, G' was in line with these results. Also, the findings are consistent with studies of model emulsion-filled polymer gels (Fuhrmann et al., 2020a; Fuhrmann et al., 2020b). We report for the incorporation of oil droplet clusters into polymer gels a 1.6-times (weak clusters) and a 3-times (strong clusters) increase of the stiffness (Fuhrmann et al., 2020a,b). The higher increase observed for emulsion-filled particle gels compared to model polymer emulsion-filled gels relates to the lower stiffness of the fat-free matrix. Similar to our previous work, we see that strong clusters provide a higher increase of stiffness than weak clusters.

Clustering of dispersed oil droplets led to a strong increase in brittleness and an earlier onset of yielding. Both cross-over strain and critical strain (10% deviation from the LVE), considerably decreased with clustering of oil droplets in processed emulsion-filled particle gels. It is known that the brittleness of emulsion-filled gels increases with increasing oil volume fraction (Sala, van Aken, Stuart, & van de Velde, 2007b). This means that the clustering of oil droplets indeed leads to an increase in effective oil volume fraction in the samples. We see that processed emulsion-filled particle gels with strong clusters for both milk fat and sunflower oil (SFO GSE and MF GSE) show a stronger increase in brittleness compared to the weaker clusters (SFO Gel-DAT and MF Gel-DAT).

Fat-reduced processed emulsion-filled particle gels with weak electrostatic clusters (MF Gel-DAT lf) (20% fat content) were as stiff as the non-clustered full-fat samples (MF non-C (29% fat content). The fat-reduced gel (MF Gel-DAT lf) also showed a comparable cross-over strain and critical strain as the full fat clustered gel sample.

### 6.3.3 Texture properties of processed emulsion-filled particle gels with and without oil droplet clusters

Table 4 summarises the instrumental texture properties of the 7 samples.

**Table 4. Texture parameters (± SD) of the samples obtained by TPA. Different letters indicate the statistical significance of the between samples (p<0.05).**

| | Emulsion-filled gel | Code | Hardness (g) | Cohesiveness (%) | Springiness (%) | Adhesiveness (g.sec) |
|---|---|---|---|---|---|---|
| *Fat-free matrix* | - | - | 56.8 ± 2.3g | 39.9 ± 3.5d | 78.7 ± 1.9e | -195.8 ± 17.5b |
| Sunflower oil | | | | | | |
| *Non-Clustered* | Non-clustered, sunflower oil | SFO non-C | 181.6 ± 0.1e | 56.6 ± 0.1c | 82.4 ± 0.1cd | -325.6 ± <0.1c |
| *Clustered* | Heteroaggregated (Gelatine-DATEM), sunflower oil | SFO Gel-DAT | 238.2 ± 27.1d | 75.5 ± 1.4a | 88.9 ± 0.9a | -171.4 ± 2.1b |
| | Cross-linked (GSE-WPI), sunflower oil | SFO GSE | 107.3 ± 4.0f | 23.9 ± 0.1e | 37.5 ± 0.4g | -102.1 ± 14.8a |
| Milk fat | | | | | | |
| *Non-Clustered* | Non-clustered, milk-fat | MF non-C | 216.1 ± 0.3d | 54.1 ± 0.7c | 81.4 ± 0.7d | -360.8 ± 23.5cd |
| *Clustered* | Heteroaggregated (Gelatine-DATEM), milk-fat | MF Gel-DAT | 404.4 ± 26.7a | 60.5 ± 0.4b | 85.6 ± 0.1b | -397.2 ± 29.9d |
| | Cross-linked (GSE-WPI), milk-fat | MF GSE | 354.3 ± 13.2b | 26.1 ± 0.1e | 65.9 ± 0.2f | -453.3 ± 9.0e |
| | Heteroaggregated (Gelatine-DATEM), milk-fat (20% fat) | MF Gel-DAT lf | 299.0 ± 3.0c | 63.4 ± 0.2b | 84.0 ± 0.2bc | -326.3 ± 21.5c |

In line with the results reported in the section on the rheological properties, the hardness of the gels increased with the incorporation of a dispersed oil phase into the matrix, as well as with increasing solid fat content. Processed emulsion-filled particle gels with milk fat as dispersed phase were stiffer than gels with sunflower oil droplets. Similarly, the presence of a dispersed emulsion phase increased cohesiveness, springiness and adhesiveness. We suggest that incorporation of a dispersed phase and the formation of a more cohesive material is linked to the reinforcing effect of the droplets as fillers. These findings are also confirmed by the results of the critical strain and cross-over strain.

Clustering of oil droplets by weak interactions led to a considerable increase in hardness of the processed emulsion-filled particle gels (SFO Gel-DAT and MF Gel-DAT) compared to samples with non-clustered oil droplets (SFO/MF non-C), independently of fat hardness. This finding is in line with the increase in G' and Young's moduli of these gels. From these results, we conclude that these clusters are indeed reinforcing the network. For samples with liquid oil (SFO GSE), the introduction of large, strong emulsion droplet clusters, however, led to a considerable decrease in hardness compared to non-clustered oil-based (SFO non-C). We observed that the deformation during the TPA led to yielding of the sample rather than the occurrence of a peak force. This suggests that clusters lead to an earlier onset of the flow of the product and lower overall network connectivity. For milk-fat based emulsion-filled gels with strong clusters (MF GSE), hardness did not decrease compared to non-clustered emulsion-filled gels (MG non-C). One explanation for this behaviour might be the occurrence of milkfat cluster jamming during deformation.

The attributes cohesiveness and springiness depended on the cluster strength. Processed emulsion-filled particle gels with both milk fat- and sunflower oil-based with weak clusters (SFO/MF Gel-DAT) showed a significant increase (p<0.05) in cohesiveness and springiness. This suggests that weak clusters indeed assist in the formation of a cohesive structure. In the case of gels with strongly interacting clusters (SFO/MF GSE),

these parameters decreased compared to the non-clustered sample (SFO/MF non-C). This indicates that these clusters might have disturbed network formation, leading to a higher matrix strength in the elastic deformation regime, but a quick structure breakdown once the material is plastically deformed. For the parameter adhesiveness, we found a strong dependency on the solid fat content. Gels with liquid oil were overall less adhesive than gels with solid milk fat. The reason for this might be the release of (liquid) oil droplets during compression, which lubricated the probe and thereby hindered the adhesion to the surface of the probe.

The fat-reduced processed emulsion-filled particle gels (MF Gel-DAT lf) showed a slightly higher hardness compared to the full-fat non-clustered gel (MF non-C), even though the fat content was reduced by 30%. This result shows once more that clustering indeed increases the effective oil volume fraction efficiently. Similarly, the cohesiveness and springiness of the fat-reduced processed emulsion-filled particle gels (MF Gel-DAT lf) increased compared to the full-fat samples with non-clustered oil droplets (MF Gel-DAT). Thus, similar to the rheological properties, these TPA results suggest that incorporation of weakly interacting oil droplet clusters can help to engineer food texture properties.

### 6.3.4 Sensory properties of processed emulsion-filled particle gels with and without oil droplet clusters

The rheological characterisation of the studied samples revealed that clustering of milk fat droplets into weakly interacting clusters could allow to engineer the rheological food properties. To determine whether clustering of dispersed fat-phase also affects sensory properties such samples, we compared the sensory profiles of the different processed emulsion-filled particle gels and, in specific, of the fat-reduced processed emulsion-filled particle gels with weakly interacting oil droplet clusters of milk fat (MF Gel-DAT lf) to a full-fat processed emulsion-filled particle gel with non-clustered oil droplets (MF non-C).

The results of the RATA sensory profiling of the 7 samples are summarised in Table 5. A PCA is provided in Figure 4, which summarises the positioning of the samples in their sensory space. A bar chart summarising the ratings of the samples for 4 key attributes, including oily, creamy, grainy and hard, are provided in Figure 5.
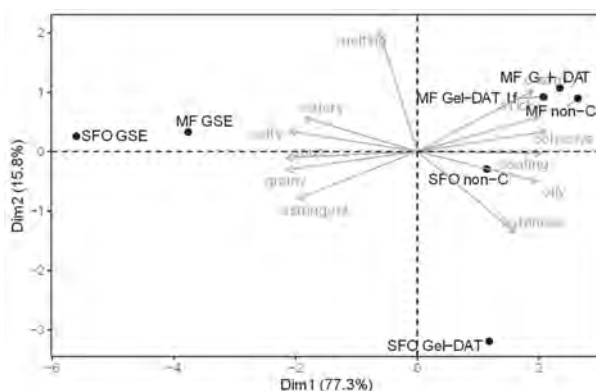


**Figure 4. PCA biplot of sensory attributes of processed emulsion-filled particle gels. The first principle component explains 77%, the second component explains 16%.**
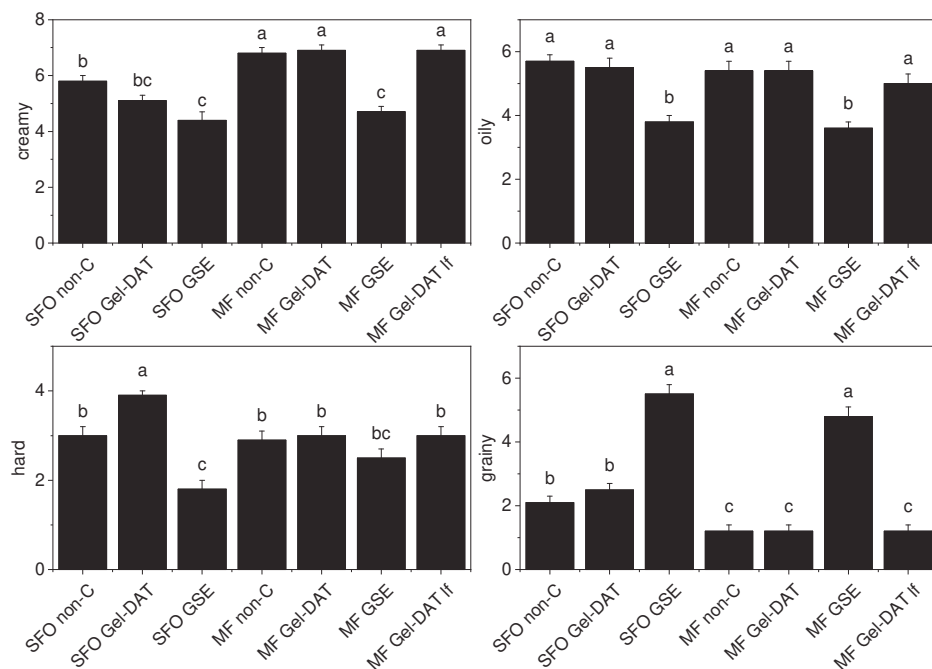
**Figure 5. Bar chart comparing selected sensory ratings for the attributes oily, creamy, grainy and hard of the different samples. Letters are indications of statistical significance of the differences (p<0.05). SFO: sunflower oil, MF: milk fat, non-C: non clustered, Gel-DAT: hetero-aggregated clusters with gelatine and DATEM, GSE: strong, cross-linked clusters by polyphenols.**

**Table 5. Summary of RATA data (n=83) of 7 processed emulsion-filled particle gels. Results are shown as average rating ± standard error of mean (SEM) with indications of significant differences between samples per attribute as different letters (p<0.05).**

| Attributes | F-value (all p<0.01) | Sunflower oil | | | Milk fat | | | |
|---|---|---|---|---|---|---|---|---|
| | | Non-clustered | Clustered | | Non-clustered | Clustered | | |
| | | Non-clustered, sunflower oil | Heteroaggregated sunflower oil | Cross-linked, sunflower oil | Non-clustered, milk-fat | Heteroaggregated, milk-fat | Cross-linked, milk-fat | Heteroaggregated, milk-fat (20% fat) |
| | | SFO non-C | SFO Gel-DAT | SFO GSE | MF non-C | MF Gel-DAT | MF GSE | MF Gel-DAT lf |
| astringent | F: 24.0 | 2.8 ± 0.2 bc | 3.0 ± 0.3 b | 4.1 ± 0.3 a | 1.9 ± 0.2 d | 1.8 ± 0.2 d | 3.3 ± 0.2 b | 2.2 ± 0.2 cd |
| coating | F: 9.4 | 5.4 ± 0.2 a | 5.0 ± 0.3 a | 4.0 ± 0.2 b | 5.4 ± 0.2 a | 5.0 ± 0.2 a | 4.0 ± 0.2 b | 5.2 ± 0.3 a |
| cohesive | F: 7.2 | 4.5 ± 0.2 ab | 4.6 ± 0.3 ab | 3.6 ± 0.3 c | 5.1 ± 0.3 a | 5.0 ± 0.3 a | 4.0 ± 0.3 bc | 4.9 ± 0.2 a |
| creamy | F: 40.8 | 5.8 ± 0.2 b | 5.1 ± 0.2 bc | 4.4 ± 0.3 c | 6.8 ± 0.2 a | 6.9 ± 0.2 a | 4.7 ± 0.2 c | 6.9 ± 0.2 a |
| grainy | F: 85.4 | 2.1 ± 0.2 b | 2.5 ± 0.2 b | 5.5 ± 0.3 a | 1.2 ± 0.2 c | 1.2 ± 0.2 c | 4.8 ± 0.3 a | 1.2 ± 0.2 c |
| hardness | F: 13.6 | 3.0 ± 0.2 b | 3.9 ± 0.3 a | 1.8 ± 0.1 c | 2.9 ± 0.2 b | 3.0± 0.2 b | 2.5 ± 0.2 bc | 3.0 ± 0.2 b |
| melting | F: 8.6 | 5.1 ± 0.2 a | 4.0 ± 0.2 b | 5.4 ± 0.2 a | 5.3 ± 0.2 a | 5.5 ± 0.2 a | 5.7 ± 0.2 a | 5.5 ± 0.2 a |
| oily | F: 17.0 | 5.7 ± 0.3 a | 5.5 ± 0.2 a | 3.8 ± 0.3 b | 5.4 ± 0.2 a | 5.4 ± 0.3 a | 3.6 ± 0.2 b | 5.0 ± 0.3 a |
| salty | F: 13.1 | 4.5 ± 0.2 b | 4.3 ± 0.2 b | 5.7 ± 0.2 a | 4.3 ± 0.2 b | 4.1 ± 0.2 b | 5.5 ± 0.2 a | 4.6 ± 0.2 b |
| sour | F: 38.3 | 4.3 ± 0.3 b | 4.1 ± 0.3 b | 6.3 ± 0.3 a | 3.3 ± 0.2 c | 3.5 ± 0.3 bc | 5.6 ± 0.3 a | 3.9 ± 0.2 bc |
| sticky | F: 16.0 | 4.5 ± 0.3 a | 3.5 ± 0.2 bc | 2.7 ± 0.2 d | 4.4 ± 0.3 a | 4.2 ± 0.3 ab | 2.9 ± 0.2 cd | 4.5 ± 0.3 a |
| watery | F: 5.2 | 3.0 ± 0.2 ab | 2.6 ± 0.2 b | 3.7 ± 0.2 a | 2.9 ± 0.2 b | 2.8 ± 0.2 b | 3.1 ± 0.2 ab | 2.6 ± 0.2 b |

From the PCA, we can see that the first two principal components explain 93% of the variation. The primary separation occurs along with the first principal component going from graininess/astringency/sourness to

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creaminess/stickiness/coating. The second principal component separates the samples from melting to hardness. Samples with large, strong clusters (SFO/MF GSE) are thereby on the far left, towards a watery and grainy perception. Milk-fat based gels (MF Gel-DAT, MF Gel-DAT lf and MF non-C), except for the gels with strong clusters, are grouped towards a creamy and cohesive perception. Sunflower oil samples seem to be characterised by an oily perception.

In terms of fat-related sensory perception, a considerable influence of clustering can be observed, as reported earlier for model o/w emulsions and emulsion-filled gels (Fuhrmann et al., 2019b; Fuhrmann et al., 2020a). We can see in Figure 5 that for samples with sunflower oil, creaminess was overall lower compared to milk fat based samples. Processed emulsion-filled particle gels with weak clusters (SFO/MF Gel-DAT) were not different in creamy perception from the relevant non-clustered gels. The incorporation of strong clusters (SFO/MF GSE) largely reduced creaminess perception. We hypothesise that these clusters entrap the fat-phase so strongly that it cannot be perceived during oral processing. In line with this reasoning, it has been described earlier that the interaction between oil droplets and matrix affect the fat-related perception considerably, with bound oil droplets reducing fat-related sensory properties (Sala et al., 2008). Thus, a similar trend was seen in the oiliness perception for gels with strong clusters (SFO/MF GSE) compared to other model foods. It is of interest that none of the fat-related sensory attributes (coating, creamy, melting, oily; see Table 4 and Figure 4) of the fat reduced (-30% fat) processed gels with weak clusters (MF Gel-DAT lf) were perceived significantly different ($p<0.05$) from the full-fat samples (MF non-C). This demonstrates that clustering is a versatile tool in engineering the food structure, to tune mechanical, but also relevant sensory properties.

In terms of texture-related attributes, we can see that perceived hardness of the gels depended on both clustering of oil droplets and the type of dispersed fat used. For sunflower oil-based gels, the hardness increased for samples with weak clusters (SFO Gel-DAT), compared to non-clustered gels (SFO non-C). The incorporation of strongly interacting clusters (SFO GSE) decreased perceived hardness compared to non-clustered samples (SFO non-C). The lower perceived hardness might be linked to the reduced cohesiveness.

For milk fat-based particle gels (MF non-C, MF Gel-DAT and MF GSE) the perceived hardness remained overall similar. Also, after the reduction of fat (MF Gel-DAT lf), perceived hardness was comparable to the full-fat non-clustered gels (MG non-C). The sample with strongly interacting clusters (MF GSE) showed, in line with the sunflower oil-based gel, a (non-significant) trend towards a lower hardness. These findings suggest that perceived hardness of the different processed emulsion-filled particle gels was an interplay of stiffness and cohesiveness. For overall less stiff gels (sunflower oil), differences in hardness were easier to be perceived than for overall stiffer samples (milk fat).

Model gels containing large and strongly interacting oil droplet clusters (MF GSE) and (SFO GSE) were perceived as significantly more grainy compared to gels with non-clustered droplets (MF non-C/SFO non-C) or samples with weak clusters (MF Gel-DAT/SFO Gel-DAT), independently of fat type. The increase in graininess was linked to the cluster size and cluster density. From the CSLM micrographs (Figure 2) it can be seen that cross-linked (GSE) clustered oil droplet structures remained large after incorporation into the processed matrix. It is known that hard particles of large sizes, *i.e.* tens of micrometres, can be detected in the mouth leading to a perception of a gritty or grainy mouthfeel (Imai, Hatae, & Shimada, 1995). Strongly interacting protein-stabilised oil droplet clusters crosslinked with GSE do not disintegrate during

oral processing and give rise to sensations of solid particles. The link between particle properties and perceived graininess is in agreement with earlier published research on the perceivability of grainy or gritty perception (Appelqvist, Cochet-Broch, Poelman, & Day, 2015; Imai et al., 1995; Shewan, Stokes, & Smyth, 2020).

To conclude, the emulsion droplet distribution and interactions in processed emulsion-filled particle gels strongly affected their sensory properties. Weakly interacting clusters were shown to allow to maintain the perception of texture- and fat-related attributes while reducing the fat content. In contrast, incorporation of strongly interacting oil droplet clusters in processed particle gels gives typically rise to undesired graininess sensations and reduces cohesiveness.

## 6.4 Conclusions

Rheological and texture properties of processed emulsion-filled particle gels are strongly affected by the incorporation of oil droplet clusters into the matrix and the type of lipid used (solid milk fat vs liquid sunflower oil). A higher solid fat content leads to stiffer gels. Clustering of oil droplets increase Young's and storage moduli and decreases critical strain, with stronger, larger clusters leading to more brittle and less cohesive samples. The incorporation of weakly interacting, small oil droplet clusters show a trend, suggesting that clustering might have beneficial effects on the fat-related sensory properties. In contrast, incorporation of strongly interacting oil droplet clusters in processed emulsion-filled particle gels typically gives rise to undesired graininess sensations and reduces cohesiveness.

Although in our study gels with liquid oil were less hard than milk fat-based gels, our results show that clustering of oil droplets also allows to obtain comparable structures in emulsion-filled gels with liquid oil as milk fat versions. Overall, this research shows that clustering of emulsion droplets is a suitable strategy to engineer food structure, allowing to tune texture and sensory properties. When applying the clustering, clusters must remain small and weak, in order to avoid a negative impact on the sensory profile.

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# Chapter 7 - Rheological behaviour of attractive emulsions with tuneable droplet-droplet interactions



| repulsive o/w emulsion | attractive o/w emulsions | | |
|---|---|---|---|
| *electrostatic repulsion* | *hetero-aggregation* | *electrostatic bridging* | *crosslinking* |

interaction strength ↑
elastic modulus G'↑

$\varphi_{oil}<0.7$: G''>G'
$\varphi_{oil}>0.7$: G'>G''

$\varphi_{oil}>0.4$: G'>G''

soft glassy behaviour

gel-like behaviour
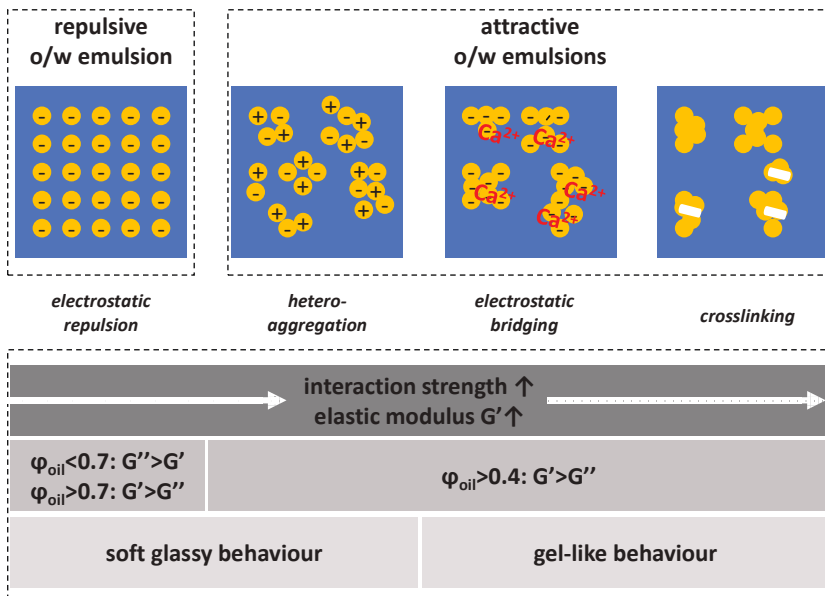
## Abstract

Interparticle interactions in colloidal dispersions can strongly affect their rheological behaviour. We investigated the effect of attractive inter-droplet interaction strength on the rheological properties of concentrated emulsions ($\varphi$=0.4-0.8) using small and large amplitude oscillatory shear (LAOS) measurements. The interaction strength was changed by varying the type of attractive interactions among oil droplets. Weak interactions were obtained using the electrostatic attraction of oppositely-charged emulsion droplets, medium-strong interactions were obtained by salt bridging with calcium and strong interactions by protein-proanthocyanin interactions. An emulsion stabilised with whey protein isolate (WPI) was used as a reference repulsive emulsion. Emulsions with strong interactions showed a monotonous yielding behaviour independent of volume fraction. Emulsions with weak interactions among droplets exhibited in an amplitude sweep apparent two-step yielding and in Lissajous plots a continuous yielding behaviour at volume fractions below maximum packing. At volume fractions above maximum packing, an overshoot in the loss modulus, G", was observed, typical for soft glassy systems above maximum packing. In emulsions with medium-strong interactions, the two-step yielding in the amplitude sweep was considerably reduced, as was the overshoot in G''. The yielding behaviour in emulsions is determined by a complex interplay of network rupture and reformation, rearrangement of clusters and cage breaking, which in turn is influenced by interaction type and strength. Investigation of the intracycle stress response revealed that above random close packing, emulsions with weak attractive interactions yield by breaking free of clusters or cages and the temporary formation of new clusters, thus indicating a soft glassy state. Strong interactions among droplets lead to apparent strain-hardening, indicating that droplet clusters undergo jamming or densification and behave as gel-like systems. These results show that small changes in the interactions between oil droplet can lead to a variety of different material properties.

## 7.1 Introduction

Many food products can be considered oil-in-water emulsions; examples include milk, dairy drinks or mayonnaise (Dickinson, 2015). The composition and structure of these foods influence their rheological properties (Fischer & Windhab, 2011). At low volume fractions ($\varphi < 0.1$) of oil droplets, emulsions behave as Newtonian liquids. When the oil fraction increases ($\varphi > 0.1$), droplet-droplet contact can occur, and the emulsions show non-Newtonian flow behaviour. With further increasing volume fractions, the shear viscosity sharply increases due to the packing of the droplets. Eventually, the oil droplets approach a maximum packing fraction ($\varphi_{max}$), and yielding behaviour appears. The theoretical packing fraction is 0.64 for random close packing and 0.74 for hexagonal packing for non-interacting hard spheres. When the droplets are deformable (*i.e.* when their Laplace pressure is relatively low), the oil volume fraction can be increased above the maximum packing fraction. In this case, the emulsion starts to show elastic properties, with a strong dependence of the elasticity on interface properties. In the case of interactions between the droplets, the maximum packing fraction can deviate. In the case of repulsive droplet-droplet interactions, the maximum packing fraction decreases as the droplets start to repel each other, thereby increasing their effective radius (Mondain-Monval, Leal-Calderon, Phillip, & Bibette, 1995). In the case of attractive interactions, the maximum packing fraction also decreases, as oil droplets start to cluster. The attractive droplets can form a network already at lower volume fractions. As a result, the effective oil volume fraction is considerably increased and the dispersions display elastic properties at absolute volume fractions below random close packing (Erramreddy & Ghosh, 2015).

The rheological properties of emulsions, and specifically their elastic and viscous behaviour, are strongly affected by the interactions among the oil droplets. For repulsive emulsions, an increase in oil volume fraction leads to a rheological behaviour where the storage modulus dominates over the loss modulus, indicating a predominantly elastic behaviour. We refer to emulsions with repulsive droplet-droplet interactions as repulsive emulsions, as also suggested in literature (Hermes & Clegg, 2013). With increasing shear rate, both moduli decrease due to network breakage and droplet alignment, and a predominantly viscous behaviour prevails. In the case of concentrated emulsions with purely repulsive interactions, the viscous modulus can exhibit a peak at a given strain (Datta, Gerrard, Rhodes, Mason, & Weitz, 2011), which is related to the temporary jamming and the following structural relaxation of the oil droplets (Koumakis & Petekidis, 2011).

When emulsion droplets experience attractive interactions, referred to as attractive emulsions, clustering of droplets occurs (Fuhrmann et al., 2019). In some cases, a two-step yielding behaviour of the viscous modulus, visible as two peaks, has been described (Datta et al., 2011). Similar behaviour was also shown for microgel particle systems flocculated because of depletion interactions and particles adsorbed at the air-water interface (salt-based screening of charges) (Koumakis & Petekidis, 2011; Zhang, Yu, Cayre, & Harbottle, 2016a). It was suggested that this two-step yielding relates to the occurrence of two rearrangement phenomena. In the first yielding process, interactions and bonds among particles are assumed to be disturbed. The first peak relates to the initial breakdown of interparticle interactions. Although fewer interactions are then present among the droplets, droplets would be still immobilised. In the second yielding step, droplets are suggested to experience a temporary stiffening by rearrangement, followed by a collapse of the structure. Thus, the occurrence of a two-step yielding phenomenon is linked,

in literature, to the co-existence of two different time scales or structural relaxation processes and two specific length scales – droplet-droplet interactions and cage characteristics (Zhou et al., 2014). Studies on microgel particle dispersions and particles at an interface have shown that the two-step yielding process only occurs in a limited range of volume fractions of attractive particles (Zhang et al., 2016a; Zhou et al., 2014). At relatively low volume fractions, no yielding behaviour was observed, and at too high volume fractions a one-step yielding behaviour is found. Two important factors remain unclear. Firstly, the role of the droplet-droplet interaction strength on the yielding behaviour of o/w emulsions is not known. Secondly, many investigations did not consider information gained from large amplitude sweeps (LAOS). However, by including these measurements, non-linear behaviour might be better understood. By studying these factors, one might obtain insights which could help to obtain emulsion-based systems with desired rheological properties. To do so, we varied the droplet-droplet interactions by using different types of interactions: (i) electrostatic attraction, (ii) salt bridging and (iii) crosslinking of protein-stabilised oil droplets with polyphenolic compounds. To characterise our systems, we performed oscillatory shear rheology measurements at small- and large-amplitudes.

## 7.2 Materials and methods

### 7.2.1 Materials
Whey protein isolate (WPI, BiPRO) was obtained from Davisco (Le Sueur, MN, USA). Gelatine (type A) was acquired from Rousselot (Son, The Netherlands). Diacetyl tartaric acid ester of mono- and diglycerides (DATEM) was kindly provided by CP Kelco Inc (Atlanta, GA, USA). As a source of polyphenols, grape seed extract (Vitaflavan®) produced by Les Dérivés Résiniques et Terpéniques (Dax, France) was used. Sunflower oil was obtained from a local supermarket. $CaCl_2$, HCl, NaOH, citric acid and disodium hydrogen phosphate were obtained from Sigma Aldrich (St. Louis, USA). For all experiments, demineralised water was used (MilliQ® system, Merck Millipore, Germany).

### 7.2.2 Methods

#### 7.2.2.1 O/w emulsions

*(Non-clustered) o/w emulsions with repulsive droplet-droplet interactions ("repulsive emulsions")*
To obtain o/w emulsions with 40% (v/v) oil, an aqueous phase containing 7.5 g/L WPI in water (pH 6.8) was prepared. The mixture was stirred overnight at 4°C, and the pH was adjusted (1M HCl/NaOH) if required. After addition of the oil phase, the mixture was pre-emulsified with a rotor-stator homogeniser (Ultra-Turrax, IKA, Germany) at 8000 rpm for 3 min. The pre-emulsions were homogenised four times (LabhoScope, Delta Instruments, The Netherlands) at 180 bar. Emulsions with higher oil volume fractions were obtained by centrifugation as described in the section "Increase in oil volume fraction".

*Preparation of o/w emulsions with attractive droplet-droplet interactions ("attractive emulsions")*
WPI-stabilised o/w emulsions with polyphenol-induced clustering
For the aqueous phase, WPI (7.5 g/L) was dissolved in a 0.12 M McIlvaine buffer at pH 3 overnight. The pH was adjusted with 1M HCl if required. Sunflower oil (40% v/v) was added to the aqueous phase. The emulsion was homogenised, as described earlier. After homogenisation, 0.75 g GSE per gram of emulsifying protein was added as an aqueous stock solution of grape seed extract (GSE) of 200 g/L GSE in

pH 3 McIlvaine buffer. Dilution due to the addition of this solution was considered negligible. After addition of the GSE solution, the emulsions were shaken and stored at room temperature for 24 hours before further use. Emulsions with higher oil volume fractions were obtained by centrifugation of emulsions as described in "Increase in oil volume fraction". Clustering was induced before concentrating the emulsions.

### Hetero-aggregated o/w emulsions

O/w emulsions with 40% (v/v) oil and with hetero-aggregated clusters were prepared by combining a non-clustered WPI-stabilised and a non-clustered gelatine-stabilised emulsion. O/w emulsions with gelatine were made by preparing an aqueous phase containing 9.6 g/L gelatine in water. The gelatine dispersion was heated in a water bath at 80°C for 30 min. At room temperature, the pH was set to pH 5 (1M HCl/NaOH). After the addition of the oil phase (40% (v/v)), the mixture was pre-emulsified and homogenised as described earlier. O/w emulsions with WPI were prepared by dissolving WPI (6.4 g/L) in an aqueous phase. The emulsion was then prepared as described. The non-clustered emulsions were stored at room temperature for 24 hours. Hetero-aggregated emulsions were obtained by combining the two non-clustered emulsions at a volumetric ratio of 1:1. After mixing, the emulsions were stored for 24 hours before further use. Emulsions with higher oil volume fractions were obtained by centrifugation mixed of emulsions as described in "Increase in oil volume fraction".

### $Ca^{2+}$-clustered o/w emulsions

A WPI-stabilised emulsion (7.5 g/L) with 40% (v/v) oil was prepared as described earlier. To induce flocculation, an aqueous solution of $CaCl_2 \cdot 2H_2O$ (0.74 g/mL) was prepared and added to the emulsions to obtain final salt concentrations between 0.01-0.26 mol/L. Samples were mixed and stored for 30 min before further use. Emulsions with higher oil volume fractions were obtained by centrifugation of emulsions as described in "Increase in oil volume fraction". Clustering was induced before concentrating the emulsions.

### *Increase in the oil volume fraction of the emulsions*

To achieve higher oil volume fractions, the emulsions were concentrated by centrifugation (Beckmann, Avanti J-26 XP, Beckman Coulter B.V., Mijdrecht, The Netherlands) for 30 min at 20,000 rpm and subsequently diluted with the corresponding aqueous phase (without emulsifier). At these centrifugation conditions, we did not find effects of centrifugation on the droplet size.

### 7.2.2.2 Physical characterisation of o/w emulsions

### *Rheological characterisation*

Rheological measurements were conducted with an Anton Paar 302 Rheometer (MCR 302, Anton Paar GmbH, Austria). A parallel plate geometry (PP25) was used. The gap size was set to 0.5 mm. The plates were roughened to avoid wall-slip. After loading, a rest time of t = 1 min was given to allow structural relaxation of the sample before starting the measurement. Low viscous paraffin oil was added at the edges of the geometry to avoid evaporation of the water in the samples during measurement.

### Small amplitude oscillatory shear (SAOS)

SAOS measurements were used to determine the linear viscoelastic properties of the emulsions. To determine the elastic and loss (viscous) modulus in the linear viscoelastic region, an amplitude sweep test

was performed with a shear strain range of 0.01–1000%, with a logarithmic ramp. The test was performed at a frequency of 10/s, at 20 °C. Frequency sweeps at a strain within the linear range (not shown) were nearly frequency independent and showed weak power-law behaviour: $G' \sim \omega^n$, with n close to zero. We report the elastic modulus G', the loss modulus G", the critical strain, which corresponds to the strain at which a 10% deviation in the elastic modulus from the linear viscoelastic range is observed, and the cross-over point of G' and G".

### Large amplitude oscillatory shear (LAOS)

The elastic and viscous response of the emulsions in the non-linear regime were determined by LAOS measurements. The amplitude was varied in the range of 0.01–1000% at a constant frequency of 10/s, at 20 °C. Lissajous-Bowditch plots were created to describe the response of the emulsions. Following the approach of Zhang et al. (2016a, b), we show Lissajous plots in 4 regimes based on the variation of G' and G'' with strain amplitude. These regimes included a strain in the linear range of G'/G'', Lissajous plots at the yield points as well as a Lissajous plot at high strains. The intra-cycle strain stiffening behaviour (S factor) and intra-cycle shear thickening behaviour (T factor) were determined as described in literature (Precha-Atsawanan, Uttapap, & Sagis, 2018). Those factors are dimensionless numbers, which can be used to identify strain stiffening (S>0) or strain softening (S<0) or shear thickening (T>0) and shear thinning (T<0), as also suggested in literature (Giménez-Ribes, Habibi, & Sagis, 2020).

### *Determination of oil volume fraction*

The oil volume fraction of samples was estimated by determining the dry matter content of concentrated samples. Samples were dried for 24h at 120°C (Binder Oven, Binder GmbH, Tuttlingen, Germany). Samples were corrected for the theoretical amount of emulsifier. All samples were measured in triplicates.

### *Determination of oil droplet size and cluster morphology*

The particle size and particle size distribution of o/w emulsions were determined using static light scattering (Mastersizer 2000S, Malvern Instruments, Ltd., Worcester, UK). The refractive index was set to 1.47 and 1.33 for the dispersed and continuous phase, respectively (Khouryieh et al., 2015). To visualise the cluster morphology, an optical light microscope (Axioskop 2 plus, Carl Zeiss AG, Germany) equipped with a camera (Axiocam ERc 5S, Carl Zeiss AG, Germany) and Visio imaging software was used. Samples were diluted with the corresponding aqueous phase on the microscope slide.

### *Zeta Potential*

The ζ-potential of gelatine and WPI-stabilized emulsions was determined using a Zetasizer Nano ZS series (Malvern Instruments, Worcestershire, UK). Non-clustered emulsions were diluted 100 times with the emulsifier-free aqueous phase (MiliQ water). Samples were measured in triplicates at 20°C.

## 7.3 Results and discussion

### 7.3.1 Repulsive emulsions

Repulsive emulsions were obtained using WPI-stabilised o/w-emulsions (pH 6.8, -38mV). Due to the overall negative charge of the proteins, the droplets repelled each other. The effect of applied strain, γ, on the elastic modulus, G', and the viscous modulus, G'', for protein-stabilised repulsive emulsions with volume

fractions, φ ,ranging from 0.7 to 0.85 is presented in Figure 1. In Figure 2, we show Lissajous plots for stress versus strain of a repulsive emulsion at φ=0.8.
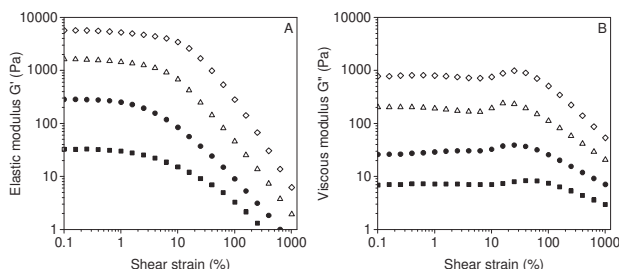


**Figure 1. Storage modulus, G', (A) and loss modulus, G'', (B) as a function of shear strain, γ, for WPI stabilised emulsion with φ of 0.70 (squares), 0.75 (circle), 0.8 (triangle) and 0.85 (diamond).**
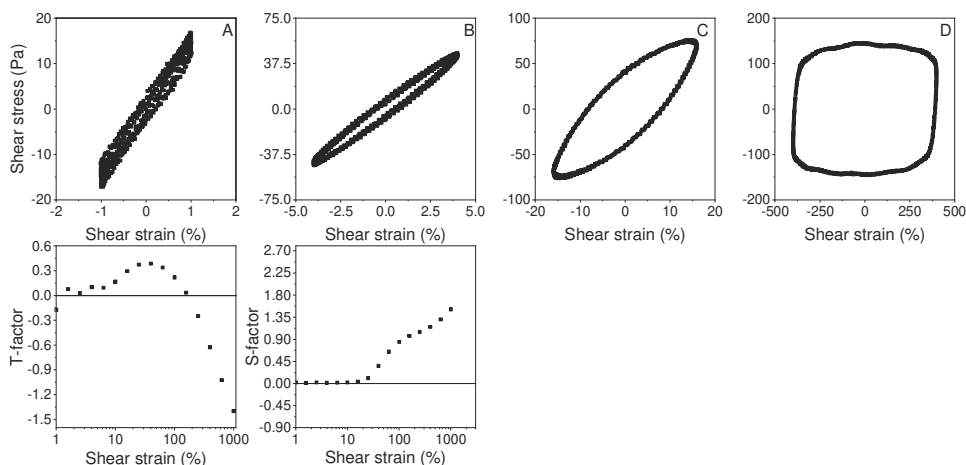


**Figure 2. Overview of LAOS measurements (shear strain vs stress) in 4 strain regions of a repulsive emulsion (0.8 oil), the strains in question are: 1% (A), 4% (B), 16% (C) and 400% (D). S- and T-factors as a function of shear strain.** For all samples, G' was initially higher than G'', revealing a solid-like nature of the samples at low strains. This elastic behaviour can also be observed in the Lissajous plots (Figure 2A), as the curve has a narrow elliptic shape. In Figure 2A, the individual loops shifted over time, leading to the unexpected vertical region at +/- 1% strain. G' increased with increasing φ, indicating that the emulsion droplets contributed to the elasticity of the network. At intermediate strains, the decrease in G' and G'' marked the onset of non-linear behaviour in the amplitude sweeps. In the Lissajous plots, the onset could be observed as a widening of the elliptic plots (Figure 2B and C). With increasing strain, emulsion behaved, therefore, as a repulsive soft glass, which has also been shown in the work of others (Tanaka, Meunier, & Bonn, 2004). The storage modulus G' increased by more than two orders of magnitude as φ increased from 0.7 to 0.85. Just beyond the maximum linear strain, G' decreased as the strain γ increased, but G'' first showed a local maximum at intermediate strain values (γ = 20-50%) before decreasing; we observe a weak strain overshoot. This

behaviour followed a, so called type III behaviour, as suggested by (Hyun, Kim, Ahn, & Lee, 2002). The emulsion network withstands the deformation up to a strain, thereby G'' increases. If this strain is exceeded, the structure cannot withstand the deformation and G'' decreases. The overall strain-softening behaviour of the emulsion can also be observed in the Lissajous plot (Figure 2D), where the curve shows a rhomboidal shape at the highest deformations. Another set of parameters which can be used to study the behaviour of materials in their intracycle behaviour are the S- and T-factors. We observe a temporary maximum in the T-factor, so the thickening ratio, which indicates shear rate thickening, followed by thinning. Due to the G'' overshoot and the maximum in the stiffening ratio, we can assume that an initial network structure was indeed present and that the emulsions did not suddenly collapse (Mezger, 2009). The emulsion droplets in such a repulsive network are present in a cage at high volume fractions. At high strains, the droplets become mobile after escaping the cage, also referred to as cage breaking (Koumakis & Petekidis, 2011; Pham et al., 2008). The overshoot in G'' was more pronounced as φ increased. With increasing φ, it is more difficult for droplets to become mobile and for the emulsion to start to flow. This behaviour has been found for repulsive dispersions (e.g. kaolin) (Bossard, Moan, & Aubry, 2007), and has also been described for solutions of large (repulsive) polymers, e.g. xanthan (Helgeson et al., 2014; John, Ray, Aswal, Deshpande, & Varughese, 2019; Mason, Bibette, & Weitz, 1995; Hyun, Kim, Ahn, & Lee, 2002). At very high strains (Figure 2D), one can observe a near plastic behaviour (Ewoldt et al., 2010). The sharp increase in stress with strain for the samples reflects the elastic deformation of trapped droplets. Then, the curves in the Lissajous plot enter a plateau region when the sample starts to flow (Shu et al., 2013). Overall, the system depicts the behaviour of a soft glassy system.

### 7.3.2 Attractive emulsions

To vary the attractive interactions among oil droplets, we made use of different interactions; (i) attractive electrostatic interactions, (ii) bridging interactions using a divalent salt and (iii) cross-linking using polyphenol-protein interactions. The effects of these different droplet-droplet interactions are discussed separately in the next sections.

### 7.3.2.1 Electrostatically clustered emulsions

The dependency of the storage modulus G' and loss modulus G'' on shear strain γ for electrostatically clustered emulsions at different oil volume fractions is presented in Figure 3 and as Lissajous plots in Figure 4.
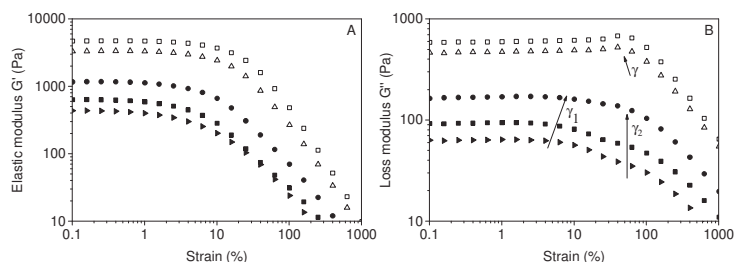
**Figure 3. Storage modulus, G', (A) and loss modulus, G'', (B) as a function of shear strain, γ, for hetero-aggregated emulsions. Oil volume fractions: 0.4 (black triangle), 0.47 (black square), 0.54 (black circle), 0.68 (open triangle), 0.77 (open square). Yielding is indicated in G'' with arrows.**
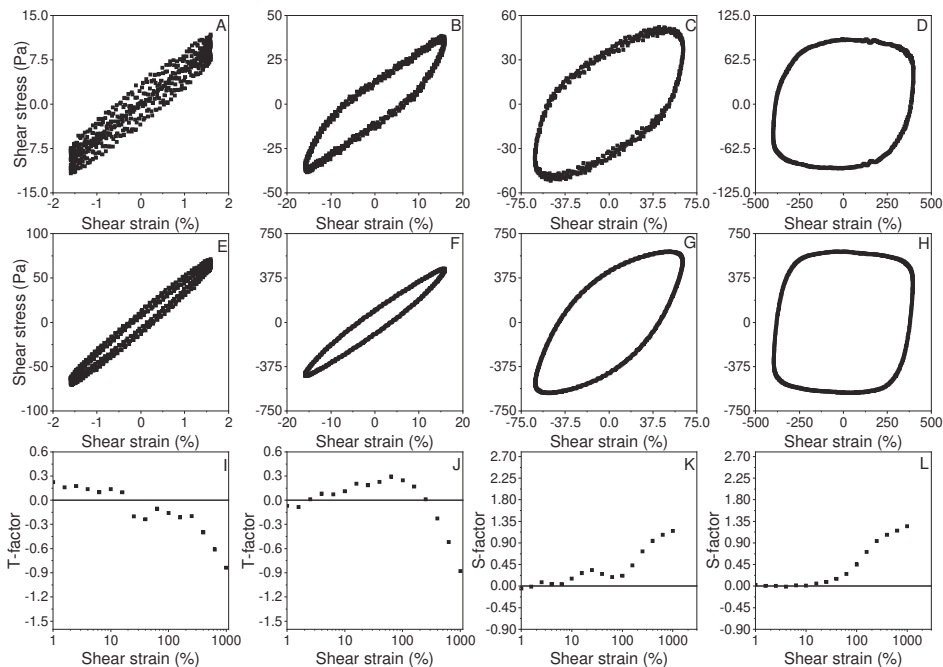


**Figure 4. Overview of LAOS measurements (shear strain vs stress) in 4 strain regions of a weak attractive emulsion (A-D at 0.47 oil) and (E-H at 0.8 oil), the strains in question are: 1.59% (A,E), 15.9% (B,F), 63.4% (C,G) and 400% (D,H). S- and T-factors of the emulsion at φ=0.47 (I, K) and φ=0.8 (J, L) are shown as a function of shear strain**

The elastic moduli, G', increased with increasing oil volume fraction, similarly to the repulsive emulsions. However, the absolute values for the storage modulus were higher for these rather weak attractive emulsions at equivalent volume fractions. The presence of inter-droplet interactions resulted in the formation of an interconnected network, which contributed to an increase in the elastic moduli of the emulsions. Due to this network formation, emulsions with predominantly elastic characteristics (at low strains) were already obtained at volume fractions below $φ_{max}$ (φ= 0.4).

For the emulsions with electrostatic interactions, in particular for oil volume fractions of 0.4 ≤ φ < 0.6, G'' displays a two-step yielding in the amplitude sweep (Figure 3). The first yield point in G'' was found at γ≈5-10% and the second at γ≈70-100%. At φ > 0.7, the two-step yielding behaviour disappeared. Instead, G'' exhibited a single maximum at strains at γ≈60%, close to the strains of the second yield point in the emulsions with φ < 0.6. The amplitude sweep, therefore, suggests that weak-attractive emulsions undergo a more complex yielding behaviour then repulsive emulsions. Such a two-step yielding process in G'' has been reported for other attractive colloidal particle systems, such as styrene and gel particle dispersions (Datta et al., 2011; Koumakis & Petekidis, 2011; Laurati, Egelhaaf, & Petekidis, 2011a; Pham et al., 2008).

The strain values at which the first yielding event occurs might be associated to the breaking of weak interactions (Datta et al., 2011; Koumakis & Petekidis, 2011; Laurati et al., 2011a; Pham et al., 2008). We can imagine that in the electrostatically flocculated emulsions, the weak interactions arise from electrostatic interactions. The breakage results in rupture of the space-spanning network (Laurati, Egelhaaf, & Petekidis, 2011b). Due to attractive electrostatic interactions, there is constant competition between bond breaking and bond reformation. At strain values below the second yielding, the reformation of the bonds to rebuild the network structure and its disruption occur at similar times scales, which delays the event of complete network breakdown. The second yielding ultimately can be associated with the breakdown of clusters due to fast structural rearrangements (Parthasarathy & Klingenberg, 1999; Pham et al., 2008) and marks the start of material flow.

The first yield point in G'' was less pronounced with increasing $\varphi$ and ultimately vanished into a one-step yielding. As the interparticle distance decreases with increasing $\varphi$, the length scales for weak bond breaking (*i.e.* the first yielding), and caging-related yielding (*i.e.* the second yielding) come closer to each other (Koumakis & Petekidis, 2011). Such concentrated emulsions exhibit a typical single peak related in literature to cage breakage (van der Vaart et al., 2013). The breakages of the weak interactions in the emulsions might still occur at lower strains. Hence, caging of particles does not only dominate the structural response and mechanical behaviour of repulsive (Koumakis & Petekidis, 2011) but also of electrostatic, attractive emulsions.

In a second step, we investigated the rheological behaviour of emulsions in the non-linear regime, by representing the LAOS measurements as Lissajous plots. Interestingly, the Lissajous plots suggested rather a gradual process of structural breakdown, where initially some residual elasticity is maintained; hence, the inclined rhomboidal shapes (Figure 4C and G). As strain increases, the plot becomes closer and closer to pure plastic behaviour, indicating that at this point, more of the structure was broken down. The T-factors suggested in the viscous part a local intra-cycle shear-thickening before overall shear thinning. S-factors displayed an increasing strain stiffening for emulsions with high volume fractions. At intermediate volume fractions the S-factor, indicating elastic nonlinearities, showed a small local intra-cycle maximum with strain-stiffening, before increasing with high shear strain. One might try to interpret this as related to the structural reorganisation in the system. However, S-factors tend to fluctuate, thus are better interpreted with some care. The difference between the amplitude sweep and the LAOS plots originates from the fact that the amplitude sweep only takes the 1st order harmonics into account. Consequently, one may wonder whether the two-step yielding behaviour in the amplitude sweep, which has been also discussed in literature (Datta et al., 2011; Koumakis & Petekidis, 2011; Zhao, Yuan, & Han, 2014), is an artefact or indeed present.

*The dependency of moduli on the interaction strength*
To investigate the effect of interactions on the yielding behaviour in more detail, we changed the degree of the electrostatic attraction among the droplets by changing the charges of the droplets of the hetero-aggregated emulsion. This was done by changing the pH. The pH was adjusted from 6.3 to 5.5 for hetero-aggregated emulsions with a volume fraction $\varphi$ of 0.4 and 0.8. The variation in pH led to a difference in zeta potential, $\Delta\zeta$, between the emulsions from 25 mV at pH 5.5 to 40 mV at pH 6.3 The G' and G'' dependency on shear strain for these emulsions is presented in Figure 5 and 6.
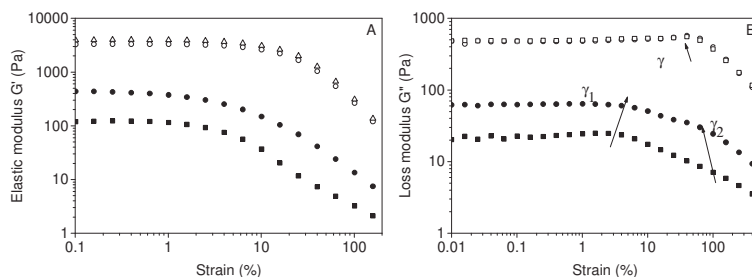
**Figure 5. Storage modulus, G', (A) and loss modulus, G'', (B) as a function of shear strain γ for hetero-aggregated emulsion with pH 5.5 (squares) and pH 6.3 (circles) at φ = 0.4 (filled symbols) and 0.8 (open symbols).**

We observed that with lower zeta potential difference, G' decreased due to lower droplet-droplet interactions. This difference was evident in the case of φ = 0.4, but the effect of interaction strength diminished for a higher volume fraction of 0.8. The reason for the relatively low impact on the elastic modulus for emulsions with higher oil volume fraction is that the system is already crowded. Particles are forced to stay in close contact with each other and caging takes place. The two-step yielding in the amplitude sweep can be still found for emulsions with lowered interactions at φ = 0.4. However, it seemed less prominent. As the clustering strength at this point was low, the emulsions hardly show resistance to complete cluster disintegration and flow.

### 7.3.2.2 Flocculation by salt-bridges emulsions

To investigate how stronger interactions affect the rheological behaviour of the dense o/w emulsions, we created droplet interactions by flocculation of oil droplets with charged emulsifiers and divalent cations. Figure 6 and 7 show the storage modulus G' and loss modulus G'' as a function of γ for protein-stabilised emulsions with salt-bridges of divalent cations ($Ca^{2+}$) and Lissajous plots of an emulsion with 0.26 mmol/mL $CaCl_2$ at oil volume fractions of 0.4 and 0.8.
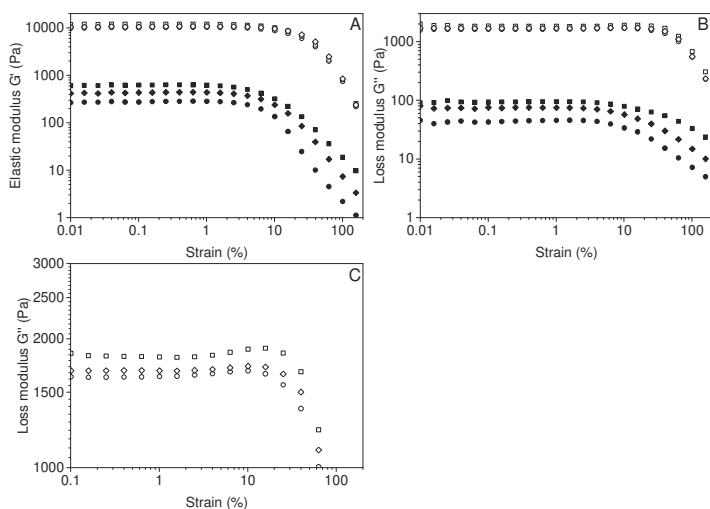
**Figure 6. Storage modulus, G', (A) and loss modulus, G'', (B) as a function of γ for emulsions clustered by salt bridges at oil volume fractions of 0.4 (open symbols) and 0.8 (filled symbols), differing in salt concentration: 0.03 mmol/mL (circle), 0.13 mmol/mL (diamond), 0.26 mmol/mL (square). A magnification of the yielding area of the loss modulus, G'', of emulsions with 0.8 volume fraction is shown (C).**
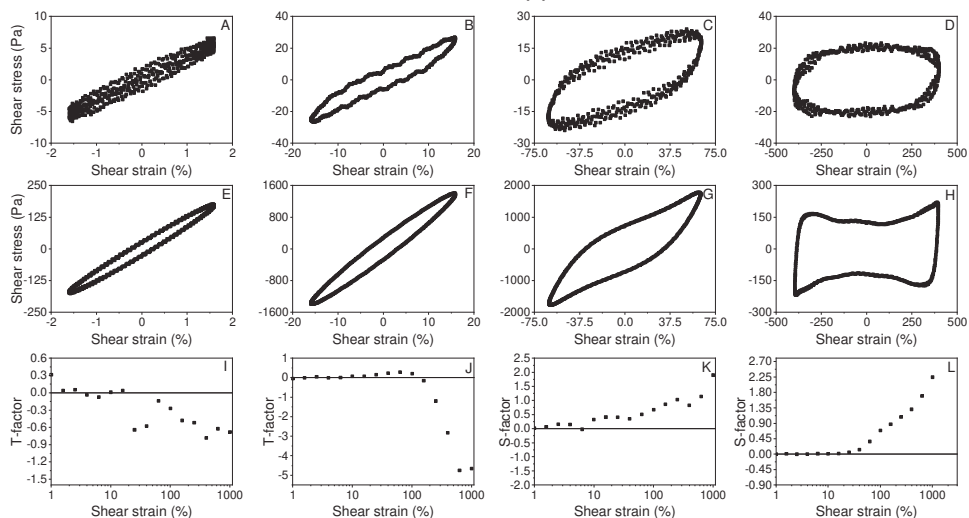


**Figure 7. Overview of LAOS measurements (shear strain vs stress) in 4 strain regions of an attractive emulsion with salt bridges (0.26 mmol/mL CaCl$_2$) (A-D at 0.4 oil) and (E-H at 0.8 oil), the strains in question are: 1.59% (A,E), 15.9% (B,F), 63.4% (C,G) and 400% (D,H). ). S- and T-factors of the emulsion at φ=0.40/0.26 mmol/mL (I,K) and φ=0.8/0.26 mmol/mL (J,L) are shown as a function of shear strain.**

As expected, an increase in G' was seen for increasing volume fractions. The G' in the linear regimes of these emulsions were higher than that of the hetero-aggregated emulsions, confirming a higher

interaction strength among the droplets of these emulsions. At φ = 0.4, the moduli displayed an explicit dependency on salt concentration. For emulsions with an oil volume fraction of 0.8, an increase in salt concentration did not lead to a substantial increase in G', in line with our findings for hetero-aggregated emulsions. One might conclude that interactions are at these volume fractions of less importance. Yet, we can observe a stress overshoot in Figure 7H, typical for gel-like behaviour. Thus, this indicates that attractive interactions do change the overall flow behaviour of the emulsion to a more gel-like system. In the absence of such interactions, a plot similar to Figure 4H or 2D would be expected. that we saw for emulsions with weaker inter-droplets interactions disappeared in samples with stronger interactions. The presence of rather strong, short and "inflexible" bonds seemingly decreased the probability of two distinct time scales of yielding and pushed the system towards a more gel-type behaviour (Blijdenstein et al., 2003; Appel, Fö, & Sprakel, 2016).

In the Lissajous plots, we observed intra-cycle strain-stiffening already at intermediate strains (Figure 7G). This suggests that the soft glassy system moved towards gel-like behaviour. Another indication of this transition from a soft glassy system to a gel-type behaviour can be seen in the Lissajous plots at high strains (Figure 7H). At high strains, the emulsion with φ>φ$_{max}$, presents a bow-tie shape, which is typically found in gel-type materials (Precha-Atsawanan et al., 2018). Thus, we conclude that by changing direct droplet-droplet interactions, the rheological behaviour of the emulsion moved towards a gel-type behaviour. The two-step yielding in the amplitude sweep that we found for weaker interacting emulsions was largely reduced.

### 7.3.2.3 Chemically-crosslinked emulsions

For emulsions with strong electrostatic bridging, we observed that the rheological behaviour moved towards a more gel-like behaviour. To confirm this transition from a soft glassy behaviour to a gel-like behaviour with increasing interactions strength, we introduced even stronger droplet-droplet interactions by crosslinking protein-stabilised droplets by polyphenol-protein interactions. The results obtained from the oscillatory strain sweep are presented in Figure 8 and Lissajous plots in Figure 9.
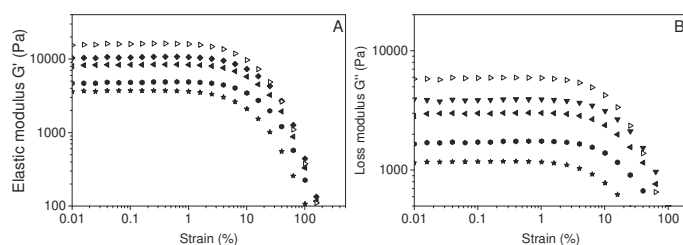


**Figure 8. Storage modulus, G', (A) and loss modulus, G'', (B) as a function of shear strain for protein-stabilised emulsions crosslinked with 0.75% GSE (g/g WPI), with φ of 0.4 (downward triangle), 0.47 (diamond), 0.58 (circle), 0.62 (square), 0.85 (triangle).**
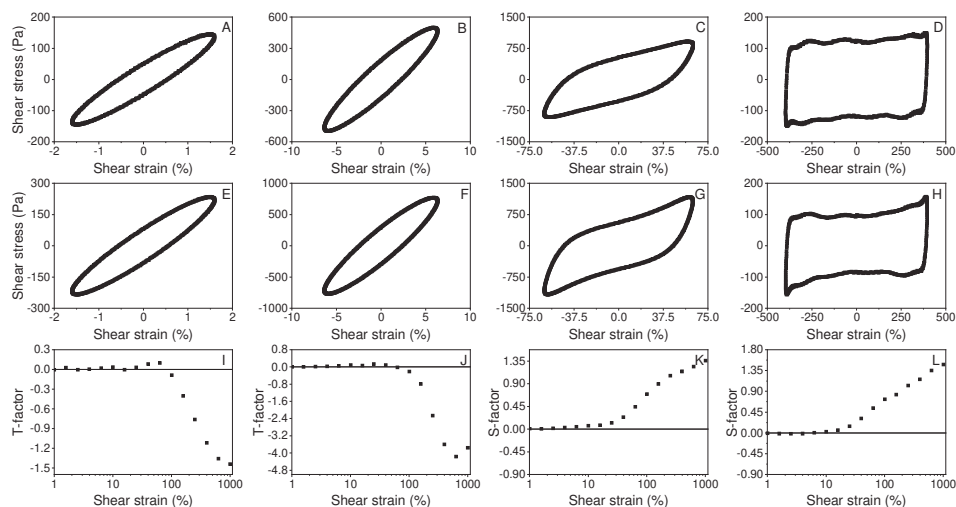
**Figure 9 Overview of LAOS measurements (shear strain vs stress) in 4 strain regions of an attractive emulsion with strong attractive interactions (A-D at 0.47 oil) and (E-H at 0.8 oil), the strains in question are: 1.59% (A,E), 15.9% (B,F), 63.4% (C,G) and 400% (D,H). S- and T-factors of the emulsion at φ=0.47 (I, K) and φ=0.8 (J, L) are shown as a function of shear strain.**

The values of G' and G'' were higher than those of the other two emulsions with attractive interactions. This was the results of a combination of different interaction types, such as electrostatic, H-bridges, as well as covalent bonds (Bohin, Vincken, Van Der Hijden, & Gruppen, 2012; Bohin et al., 2014). Interestingly, the dependency of G' and G'' on φ was less pronounced for these strongly interacting emulsions, compared to electrostatically clustered emulsions or emulsions clustered by divalent salts. This suggests that the strong inter-droplet interactions create a strong space-spanning network already at low oil volume fractions and an increase in volume fraction only has a small effect on further contributions to the emulsion elasticity. Furthermore, emulsions with strongly interacting droplets did not show a G'' overshoot above $φ_{max}$ or in the T-factor, in contrast to the emulsions with weak attractive and attractive interactions by salt-bridging. This implies a gel-like behaviour. A further indication of the transition towards a gel-type behaviour can be seen from the Lissajous plots. In Figure 9C and G, the Lissajous plots of the emulsions with strong, attractive interactions showed an upward convex nature, indicating apparent strain-stiffening (as also shown by the S-factors) after intracycle strain-softening (John et al., 2019). Even though Lissajous plots at even higher strains (Figure 9D and H) have to be examined with care, as wall slip phenomena might have occurred and inertia might play a role, the change in the shape of Lissajous curves show similarities with the upward edges observed by Zhang and co-workers for silica particle-laden interfaces (Zhang et al., 2016a). This behaviour is typically observed for samples that exhibit a gel-type behaviour and a monotonous decrease in G'' when plotted against the strain. The abrupt yielding behaviour in the amplitude sweeps and the fast decrease in the moduli can be attributed to high bond energy among the droplets (Appel et al., 2016). The high bond energy makes breakage of bonds and the occurrence of distinct yielding phenomena difficult. At both $φ<φ_{max}$ and $φ>φ_{max}$, the yielding in G'' ($γ≈$ 10%)

can be assumed to result from network disintegration rather than a step-wise breakage of clusters and cluster-cluster interactions (Laurati et al., 2011a; Pham et al., 2008).

### 7.3.3 Effect of interaction strength on rheological behaviour

Previous research suggested that attractive interactions lead to a two-step yielding behaviour of colloidal glasses due to the occurrence of two distinct relaxation phenomena (Datta et al., 2011; Koumakis & Petekidis, 2011; Pham et al., 2008). This raises the question of why such behaviour only occurred in amplitude sweeps for emulsions with weaker attractive interactions, and not for those with stronger ones.

In studies that reported a two-step yielding behaviour in amplitude sweeps, colloidal glasses, particle or droplet interactions were mostly induced by depletion interactions or by the screening of repulsive interactions (Datta et al., 2011; Pham et al., 2008; Zhang et al., 2016b). Depletion interactions, however, are the result of an exclusion process rather than of direct attraction among particles. Therefore, the interaction strength among droplets is weak, reversible and flexible (Blijdenstein et al., 2004). In contrast, the interactions investigated in this study were direct attractive droplet-droplet interactions, and, therefore, significantly stronger. Thus, they resulted in the formation of less flexible bonds. This suggests that a two-step yielding behaviour in emulsions can not only occur in a limited volume fraction range but also within a limited interaction strength range.

Interactions among droplets also affect the rheology of emulsions at $\varphi > \varphi_{max}$. For concentrated emulsions with repulsive and weak attractive interactions, we observed an overshoot in G'' and apparent strain-hardening in the amplitude sweep and the T-factors, linked to temporary densification of the droplets, before the emulsion starts to flow. Concentrated emulsions with strongly attractive interactions, however, did not show this overshoot. Based on their rheological properties, we can conclude that the emulsions with strongly attractive interactions predominantly show gel-like properties. The monotonous strain-softening behaviour in the amplitude sweep can be compared to chain orientation and alignment of the microstructure in gels (Sim, Ahn, & Lee, 2003). The presence of strong and inflexible bonds among droplets decreases the probability of network rupture and structural rearrangements of bonds. In such emulsions with strong droplet-droplet interactions, inter-cluster rearrangements are more likely to occur than intra-cluster rearrangements. It appears that droplet clusters do not get destroyed, but rather align along the flow direction, enabling flowability of the sample (Blijdenstein et al., 2004). Thus, at high volume fractions, emulsions with strong, attractive interactions behave as gels, whereas weakly attractive emulsions behave as soft glasses.

### 7.3.4 Volume fraction dependencies

To be able to better compare emulsions with weak attractive interactions (hetero-aggregated emulsions), strong, attractive interactions (clustered by GSE) and repulsive emulsions, relevant rheological parameters of the studied systems were plotted as a function of $\varphi$ (Figure 10).
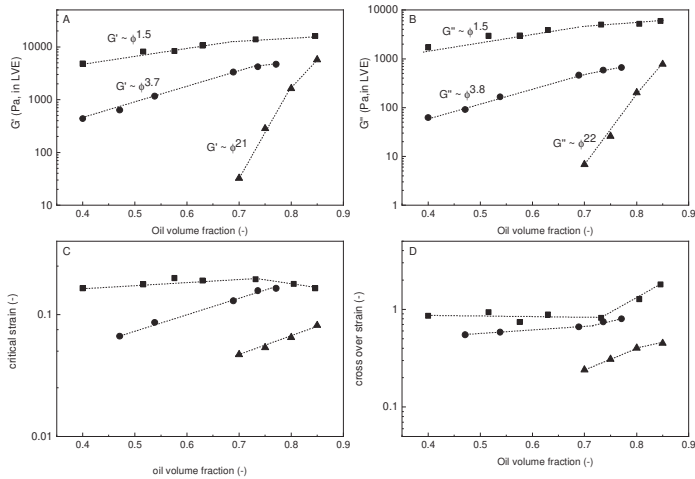
**Figure 10. Storage modulus, G', (A) and loss modulus, G'', (B) (at γ=0.1%), cross-over strain (D), critical/yield strain (C) as a function of oil φ for emulsions crosslinked by GSE (squares), hetero-aggregated emulsions (circles) and repulsive emulsions (triangles). The lines are added to guide the eye. Indications of the power-law index n of G'~φ$^n$ and G''~φ$^n$ are provided (data was fitted until φ<φ$_{max}$, all samples R$^2$>0.98)**

7.3.4.1 Modulus dependency on the volume fraction

The values for G' were lowest for the repulsive emulsions, and highest for the strong, attractive emulsions. This is likely due to the difference in cluster formation for these interaction strengths. For both emulsions with attractive interactions, generally a lower volume fraction dependence of G' and G'' was observed compared to the repulsive emulsion. This dependence became weaker as cluster strength increased. The reason for the low dependency of the moduli on oil volume fraction is that emulsions with strong, attractive emulsions behave quasi as gels. Similar scaling factors of G' have been found for suspensions of various hydrocolloids, including, *e.g.* gellan (García, Alfaro, Calero, & Muñoz, 2011). Repulsive and weak attractive emulsions behave as glassy systems, which were reported earlier to have a strong dependence of the moduli on φ (Koumakis & Petekidis, 2011; Koumakis, Schofield, & Petekidis, 2008; Lowen, 1990).

7.3.4.2 Yield strain dependency on the volume fraction

The interactions among the droplets affect the yielding behaviour of the emulsions, as the network formation is changed. The yield strain of a material consisting of clustered particles can be related to the strength of the junctions among clusters (Selway & Stokes, 2014). In our study, emulsions with strong, attractive interactions exhibited the highest yield strain values, whereas the lowest values were found for repulsive emulsions. This is consistent with results reported for hard-sphere dispersions (Priya & Voigtmann, 2014; Zong, Yuan, Zhao, & Han, 2013). The weakly attractive emulsion showed an increase in critical strain with increasing φ. Instead, strongly attractive interactions (cross-linked by GSE), showed a slight decrease in critical strain once the volume fraction exceeded φ$_{max}$. The decrease in critical strain upon approaching close packing indicates that these strongly-flocculated samples become more brittle (Koumakis & Petekidis, 2011). This might be caused by a reduction in deformability of clusters when

interparticle interactions are strong (Montesi, Peña, & Pasquali, 2004), and increased rigidity of the gel (Dickinson, 2012).

### 7.3.4.3 Cross-over point dependency on the volume fraction

For both attractive emulsions, the cross-over strain at a given φ was higher than for the repulsive emulsion, with the strongly flocculated emulsion (GSE) exhibiting highest values over the φ range. This indicates that the solid-liquid transition shifts to higher γ with increasing strength of attractive interparticle interactions. Thus, materials with a more solid character can be obtained based on the interactions of the oil droplets. We suggest that the structural rearrangement leading to flow observed with increasing deformation is less easily achieved due to strong interactions. For all emulsions, at φ > 0.7 the cross-over-strain increased with increasing φ, which was most likely due to the increasingly dense packing of droplets and encaging. At φ < 0.7, when caging effects were not yet present, the cross-over point was found to be independent of oil volume fraction for the attractive emulsions. This suggests that below $\varphi_{max}$ the liquid-solid transition is predominantly determined by the droplet-droplet interactions within the clusters and the resistance to cluster disruption.

### 7.3.5 Glass or gel?

Attractive emulsions differ in their rheological response not only from the repulsive emulsion, but also significant differences were observed between them. The rheology of such systems depends on φ and the inter-droplet interaction strength. This indicates that different classes of viscoelastic solids can be obtained. The distinction between these systems is especially difficult for different attractive emulsions at high φ, as the structure breakdown happens due to intra-cluster, inter-cluster rearrangements, as well as caging effects. These events depend on the specific interactions within and between clusters and therefore contribute to the emulsion microstructure and rheological response. Previously, it was found that two qualitatively different glasses exist, repulsive and attractive glasses (Cipelletti & Ramos, 2002). The results of this study suggest that further differentiation among attractive glassy emulsions might be appropriate. At $\varphi > \varphi_{max}$, emulsions are generally in a glassy state. However, an increase in (attractive) interaction strength can shift a glassy state to a gelled state as also proposed by Tanaka (Tanaka et al., 2004). This shift also changes the rheological behaviour, where a monotonous yielding is obtained. We can, therefore, differentiate between repulsive, weak attractive, attractive and "gelled" glasses, depending on interaction type/ strength. A schematic representation can be found in Figure 11.
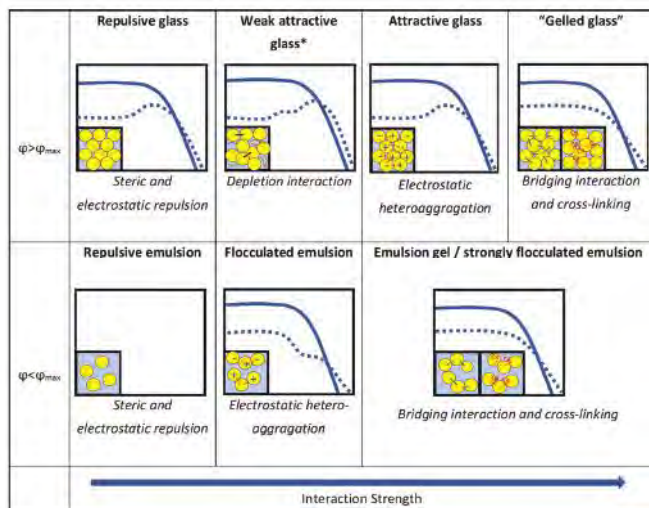
**Figure 11. Classification of emulsions depending on volume fraction and interaction strength/type. The corresponding schematic representation of G' (line) and G'' (dashed line) dependency during strain-controlled LAOS measurements is shown.**

## 7.4 Conclusions

This study aimed to understand better how attractive droplet-droplet interactions affect the yielding behaviour of concentrated emulsions. Small and large oscillatory shear measurements were performed for repulsive emulsions and emulsions with increasing attractive interaction-strength, using (i) hetero-aggregation, (ii) bridging with divalent ions and (iii) cross-linking with proanthocyanins-protein interactions. We found that the interaction strength considerably influences the rheology of the emulsion. Attractive emulsions exhibited a greater network strength than repulsive emulsions. At $\varphi < \varphi_{max}$, the rheology of strong attractive showed gel-like behaviour, while weak attractive emulsions showed a soft glassy behaviour. The dominant factor is droplet-droplet interaction strength. At $\varphi > \varphi_{max}$, caging effects were found to be the dominant factor to determine the linear-viscoelastic behaviour. Weak attractive emulsions show in the amplitude sweep a two-step yielding but in Lissajous plots a continuous yielding. The two-step yielding behaviour in G'' for attractive colloidal systems is not a general phenomenon in the case of attractive interactions in emulsions. This two-step yielding is absent in strong, attractive emulsions. These emulsions yield rather sharply, as clusters rearrange spatially and then disintegrate. LAOS measurements suggested that at $\varphi > \varphi_{max}$, weak attractive emulsions yield by cutting off loose clusters. In contrast, strong clusters undergo shear-induced jamming during yielding as indicated by temporary intracycle strain-stiffening behaviour. Overall, we conclude that the rheology of an emulsion can be tuned by manipulating the droplet-droplet interactions.

# Chapter 8 - Characterisation of friction behaviour of intact soft solid foods and food boli
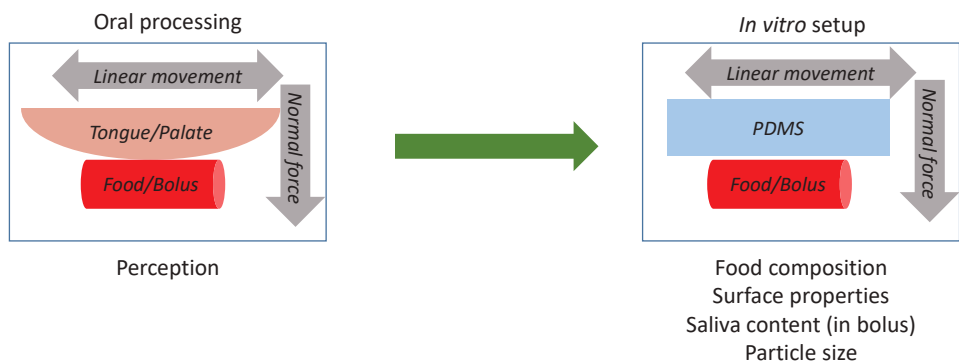
## Abstract

Methodologies to quantify friction forces between soft solid foods or food boli and (model) oral surfaces are desired to better understand how changes in food properties during oral processing affect sensory perception. In this short communication, friction forces ($F_F$) occurring between polydimethylsiloxane (PDMS) surfaces and intact soft solid foods/boli were quantified. As examples for intact foods, we used gelatine gels varying in composition and particle size, and sausages were used as an example for real foods. Friction forces measured during the relative motion of intact foods against a rough PDMS surface ("oral surface"), strongly depended on the composition of the food. Friction forces were significantly lower when PDMS against emulsion-filled gels were measured, than for unfilled gels against PDMS, likely due to the lubricating effect of released oil from the gel. Moreover, sausages, displayed significantly higher friction forces than gelatine gels when moving against the PDMS probe, presumably linked to differences in the surface of the foods. The friction forces observed for the PDMS probe moving against food boli were dependent on particle size and saliva quantity; boli with larger particle sizes showed significantly lower friction forces, whereas the addition of saliva to food boli first increased friction forces, but with increasing amount decreased the friction forces significantly. We conclude that the presented methodology can quantify the friction behaviour of intact soft solid foods and boli directly, considering (i) the effect of composition and added fillers, (ii) serum or oil release and (iii) bolus particle size.

**8**

## 8.1. Introduction

The structure of soft solid foods changes dynamically throughout its consumption; food is reduced in size, mixed with saliva and enzymes, and a cohesive mass is formed (Aguayo-Mendoza et al., 2019; Stieger & van de Velde, 2013). During this process, the mechanical properties of food change, as does the perception (Young, Cheong, Hedderley, Morgenstern, & James, 2013; Chen, 2009). In order to know processing and composition of foods affect sensory perception, the dynamic changes in the rheological and tribological behaviour of foods must be studied (Dickinson, 2018; Stokes, Boehm, & Baier, 2013). The relationships between lubrication properties and sensory perception have already been discussed for many liquid foods, including milk or o/w emulsions (Laguna, Farrell, Bryant, Morina, & Sarkar, 2017; Carvalho-da-Silva, van Damme, Taylor, Hort, & Wolf, 2013; Dresselhuis et al., 2007; Wang & Chen, 2017; Sarkar, Andablo-Reyes, Bryant, Dowson, & Neville, 2019), and gel-like foods with irreversible spreading properties, such as custards (de Wijk, Prinz, & Janssen, 2006), mayonnaises (Douaire, Stephenson, & Norton, 2014), and fluid chocolate samples (Carvalho-da-Silva, Van Damme, Taylor, Hort, & Wolf, 2013). The lubrication behaviour of such foods is often assessed in tribological setups in which the liquid food lubricates the contact area between a flat surface and a spherical probe. The surfaces are typically made of polydimethylsiloxane (PDMS), glass, steel or animal tissue (Chen, 2014; Solway & Stokes, 2013, Dresselhuis, de Hoog, Cohen Stuart, Vingerhoeds, & van Aken, 2008; Rank, Servais, Chauvy, Debaud, & Mischler, 2006; Efimenko, Wallace, & Genzer, 2002). In contrast to liquid foods, less is known about the tribological behaviour of soft solid foods moving against oral surfaces; specifically, there is a lack in approaches for the characterisation of the tribological behaviour of soft solid foods. In this case, we refer to soft solid foods as self-supporting gel-like foods with reversible behaviour (Dickinson, 2013; Vliet, 2013), such as cheeses and sausages. Some studies have been performed on soft solid foods that were reduced in size to obtain suspensions of particles to entrain those particles between two solid surfaces as a lubricant. Krop and co-workers, for example, determined lubrication properties of soft solid food boli gel particles using a PDMS ball and PDMS disk setup (Krop, Hetherington, Holmes, Miquel, & Sarkar, 2019). Liu and co-workers quantified the tribological behaviour of soft solid broken down gel particles entrained between PDMS and glass surfaces (Liu, Stieger, van der Linden, & van de Velde, 2016). To the best of our knowledge, tribological characterisation of intact soft solid foods in a mouth-mimicking setup has not been reported. However, extensive research has been performed on hydrogels prepared from non-food grade materials, such as polyvinyl alcohol, poly (2-acrylamido-2-methylpropanesulfonic acid) or poly (acrylic acid) (Gong, 2006; Gong & Osada, 2002; Oogaki et al., 2009; Zhao, Liu, & Liu, 2018). From studies on hydrogels, we know that the friction behaviour can deviate from solid materials (Rudge, Scholten, & Dijksman, 2019; Oogaki et al., 2009; Gong, 2006). Important parameters that have been shown to affect the friction behaviour include charge (Liu, Thormann, Tyrode, & Claesson, 2015), porosity (Caravia, Dowson, Fisher, Corkhill, & Tighe, 1993), substrate effects (Gong & Osada, 2002, Gong, 2006) and the presence of polymer brushes (Ishikawa, Hiratsuka, & Sasada, 2006). Furthermore, adhesion or repulsion between the probe and the gel can strongly interfere (Gong, 2006). Although many factors in hydrogels have been studied, intact food gels are largely unexplored. The difference between polymer gels and soft solid foods lies in the high compositional complexity and heterogeneity of soft solid foods. Foods consist of mixtures of proteins, polysaccharides and oil, which often have ill-defined properties and are polydisperse in nature. It would be beneficial to understand the friction behaviour of intact soft solid foods in contact with surfaces simulating oral surfaces, but also food boli at different stages of mastication (Pradal & Stokes, 2016). This

can contribute to understanding sensory perception during different stages of oral processing, such as sticky, fatty and mouth-coating sensations. This study, therefore, aimed to find an approach allowing to quantify friction forces ($F_F$) occurring between intact solid foods and food boli and simulating oral surfaces. We specifically test whether small changes in composition can be quantified by taking into account (i) addition of different dispersed particles to the gel matrix, (ii) expelled serum or oil from the matrix and (iii) effect of bolus particle size. We also incorporate saliva into the food (bolus), as this is well known to increase the oral lubricity of foods. We propose an experimental approach using a flat, cylindrical PDMS surface to mimic the oral surfaces, which is in direct contact with the intact soft solid food. For comparison, we examined intact and broken-down sausages, simulating real food boli at different stages of oral processing.

## 8.2. Materials and methods

### 8.2.1 Materials

Polydimethylsiloxane (PDMS 184 silicone elastomer kit) was bought from Dow (Dow silicones, Dow Europe GmbH, Wiesbaden, Germany). Ethanol was purchased from Sigma-Aldrich (Steinheim, Germany). Hot dog sausages (Unox, Unilever, Rotterdam, Netherlands) were kindly provided by Unilever Nederland BV. Potato starch (Honig, Amsterdam, Netherlands) and sunflower oil (Reddy, Vandermoortele, Gent, Belgium) were purchased at a local supermarket. Gelatine (from pork skin, bloom 250, isoelectric point of 8-9) was obtained from Rousselot (Gent, Belgium). Unstimulated saliva was collected as described in literature (Silletti, Vingerhoeds, Norde, & van Aken, 2007). In short, the saliva of one, generally healthy volunteer was collected, in the morning. The participant was asked to abstain from food intake for one hour before collection. Before the saliva collection, the participant was asked to rinse the mouth with water and collect saliva for one minute; this saliva was discarded. Subsequently, unstimulated saliva was collected. The saliva was centrifuged at 4°C at 10000 g for 30 min to remove debris (Beckmann, Avanti TM J-25 I, JA-21, Beckman Coulter B.V. Mijdrecht, The Netherlands) and kept on ice. Saliva was then used with no further storage. Demineralised water was used for all experiments unless stated otherwise.

### 8.2.2 Preparation of model soft solid foods and *in vitro* masticated boli

Three model soft solid foods differing in composition were prepared. The samples consisted of gels with gelatine as the continuous protein matrix and included oil droplets or starch granules as the dispersed phase. The composition, mechanical properties of the model systems and an overview of all samples used for tribological tests are shown in Table 1.

**Table 1. Overview of all intact soft solid foods and model food boli used for the tribological measurements. 1Volume of lubricant added on top of intact soft solid food was 2.5 mL. 2Concentration of saliva as a lubricant added into bolus was varied (5, 10, 20, 30 and 40% (w/w)). * Particle size of broken down soft solid food boli were 4.5 and 8.0 mm.**

| Sample | Composition | | | Mechanical properties | | | Intact soft solid food | Soft solid food bolus* |
|---|---|---|---|---|---|---|---|---|
| | Protein (% w/w) | Fat (% w/w) | Starch (% w/w) | True fracture stress (kPa) | True fracture strain (-, x100 for %) | Young's modulus (kPa) | Lubricant (on gel)[1] | Lubricant (incorporated)[2] |
| Sausage | 14 | 14 | 5 | 33.1 ± 0.5 | 0.56 ± 0.03 | 33 ± 8 | water, saliva, oil | saliva |
| Gelatine gel | 7.5 | 0 | 0 | 34.0 ± 2.0 | 1.31 ± 0.03 | 8 ± 3 | | |
| Starch-filled gelatine gel | 7.5 | 0 | 5 | 20.5 ± 2.9 | 1.12 ± 0.07 | 4 ± 1 | | |
| Emulsion-filled gelatine gel | 7.5 | 14 | 0 | 40.5 ± 1.2 | 1.13 ± 0.05 | 18 ± 1 | | |

Gelatine (12.5%) was dispersed and hydrated in cold water for 2 hrs, and subsequently melted in a water bath at 80˚C for 30 min. The pH of the gelatine dispersions was 7. Thus, the gelatine was slightly positively charged. To obtain starch-filled gelatine gels, gelatine solutions were mixed with a potato starch suspension (12.5% (w/w)). The mixture was blended using a high-speed blender for 3 min at 10,000 rpm (Digital Ultra Turrax T25, IKA Werke, Germany). The final mixture contained 7.5% (w/w) gelatine and 5% (w/w) starch. To obtain emulsion-filled gelatine gels, liquid solutions of gelatine (12.5%) were first diluted with water and then mixed with sunflower oil to reach a final concentration of 14% (w/w) oil and 7.5% gelatine. The blend was mixed with a high-speed blender for 1 min at 10,000 rpm and homogenised in three cycles at 180 bar (LabhoScope homogeniser, Delta Instruments, Drachten, Netherlands). Gelatine gels without additional components were obtained by diluting gelatine solutions with water to a final protein content of 7.5% (w/w). All warm, liquid mixtures (80˚C) were poured in Petri dishes (diameter 85 mm) to a height of 7 mm and stored at 4°C overnight to solidify. Gels were cut after solidification into cubes of 55x40x7 mm. Commercial hot dog sausages were cut along their longitudinal plane with a scalpel into cylinders of 55 mm length and 20 mm diameter.

To obtain "*in vitro* masticated" food boli, gels and sausages were ground using a meat grinder (Westmark, Lennestadt, Germany). The particle size of the boli was varied using two different pore sizes (4.5 and 8.0 mm) of the grinder to mimic boli during different stages of oral processing. Various concentrations of saliva (5, 10, 20, 30 and 40% w/w) were added to the boli. Saliva (unstimulated) was collected as described in the materials section 8.2.1. Saliva was incorporated by mixing 12 g of ground boli and the respective amount of saliva in a 60 mL syringe (BD Plastipak Luer-Lok, USA) and careful pumping the plunger 20 times; no considerable changes in particle size (4 ± 2 mm and 7 ± 3mm) were observed. This methodology is based on the procedure described by Ishihara et al. (2011).

### 8.2.3 Mechanical properties of soft solid food gels and sausages

Cylindrical pieces of gels (27 x 20 mm) and sausages (20 x 20 mm) were analysed in a Texture Analyser (TA XT plus Texture Analyser, Texture Technologies, Hamilton, USA). Gels were compressed at a rate of 1mm/s with an acrylic disk (diameter 100 mm) until 80% strain was reached. Surfaces were wetted with paraffin oil to avoid friction. Measurements were performed at room temperature (24±2˚C) in triplicate. Young's Modulus, fracture stress and fracture strain were obtained from the stress-strain curves and averaged over replicates.

### 8.2.4 Methodology to measure tribological behaviour of soft solid gels and food boli

A schematic representation of the measuring setup can be found in Figure 1. Friction behaviour was measured with a commercially available universal mechanical tester "Tribolab" (UMT, Bruker, Billerica USA). As a lower drive, a reciprocating drive (5 mm stroke length, reciprocating frequency ranging from 0.01 to 8.25 Hz) was used in combination with a holder, lined with a smooth PDMS surface (60x40x4.5 mm), facilitating the fixation of the sample in the holder (Figure 1). Intact soft solid gels were mounted in the holder and fixed with metal plates and screws to avoid slip or buckling. Thus, the tribological pair for the intact soft solid foods is PDMS/gel (Figure 1c), where the PDMS simulates oral surfaces. When the intact gel was used, the friction force was determined by the contact of the flat gel surface area with the upper probe. Broken down fragments were placed into the liquid holder without further fixation. Sufficient bolus material was added to ensure sufficient contact between the bolus material and the probe during the measurement. Thus, the tribological setup for the food boli is PDMS/food bolus particles (Figure 1b). One must consider that in this setup, two friction forces are involved, (a) the friction force between probe and bolus sample and (b) between bolus particles within the bolus.

Samples, having a height of 7mm, were brought into contact with the upper probe (rough PDMS) with a normal force of 0.5 N. A cylindrical upper probe, with curved edges, made of roughened PDMS (30 x 15 mm, 706 mm²) was designed in-house. PDMS was mixed with the supplied crosslinker in a 10 to 1 volume ratio, as reported previously (Dresselhuis, de Hoog, Cohen Stuart, & van Aken, 2008) and subsequently de-aired for 2 hrs at room temperature. A mould for the cylindrical upper probe was coated with sandpaper (size 240, corresponding to an average particle diameter of 53 μm, according to ISO 6344 (1998)) to create a rough PDMS surface. Following de-airing, the sample was cured at 60°C overnight. The modulus of the PDMS probe is about 2 MPa. A 20 N friction/load sensor (DFM-2G, Bruker, Billerica USA) was used.

The friction force ($F_F$) was subsequently measured at speeds ranging from 0.1 mm/s to 90 mm/s. Measurements were performed in triplicates, and average values with standard deviation obtained. Measurements were analysed for significant differences using a two-way ANOVA and a Bonferroni post-test with $p<0.05$. We compared means at one speed of different samples as well as differences within one sample due to sliding speed.

Additionally, intact soft solid foods were measured in the presence of lubricants (water, saliva, oil) added on top of the surface of the soft solid food. Lubricants (2.5 mL) were added with a pipet directly before the measurement onto the surface of the intact gel. By those means, the effect of the presence of lubricants on the surface of soft solid foods on friction behaviour was assessed. This was done to mimic the release of liquid from the food during mechanical testing and to mimic saliva incorporation into the food bolus during oral processing.
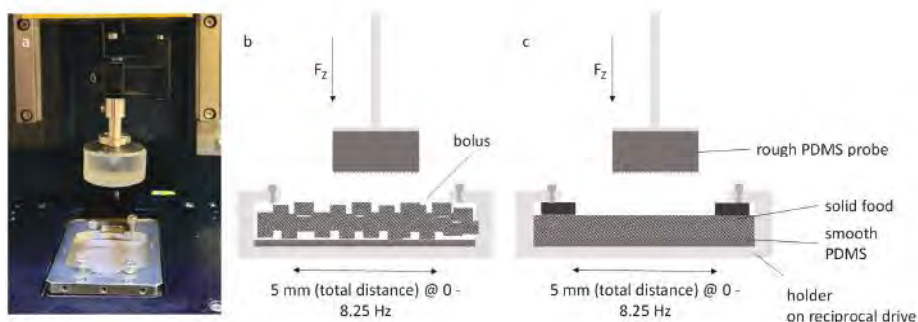
**Figure 1. Overview of the tribological setup. a: Measurement setup with intact soft solid food gel, b: schematic representation of tribological setup with broken-down food bolus particles (height bolus/gel 7 mm), c: schematic representation of the tribological set up with soft solid food.**

## 8.3. Results and discussion

The mechanical properties of the gels and sausage are given in Table 1. As can be seen, the addition of starch reduced the fracture stress, fracture strain and Young's modulus of gelatine gels, while the incorporation of oil droplets increased fracture stress and Young's modulus and decreased fracture strain. The friction force, ($F_F$), measured between the intact soft solid foods and the mouth-mimicking PDMS, is given as a function of speed in Figure 2. Friction force $F_F$ increased with increasing velocity, even to values higher than the applied normal force (0.5 N). We suggest that this is due to adhesive forces and possibly attractive electrostatic interactions between positively-charged gelatine and negatively-charged PDMS (Beal, Bubendorfer, Kemmitt, Hoek, & Mike Arnold, 2012; Gong, 2006). The friction force $F_F$ increased until a sample-dependent plateau value of around 0.6 to 0.8 N was reached. Over the entire speed range, differences in friction behaviour between gels can be observed. The friction forces $F_F$ recorded for the intact gels are related to the composition of the gels (Figure 2a). Low friction forces were obtained for emulsion-filled gelatine gels, with significant differences ($p<0.05$) between the non-filled gel and the emulsion-filled gel in the range from 1 to 10 mm/s. Starch-filled gelatine gels displayed a similar friction force as the non-filled gels (differences are not significant). The lower $F_F$ for oil-containing gels could be related to either oil release or serum release. We suggest that oil release is mostly responsible for this observation, as, after the measurements, small patches of oil were observed on the surface of the emulsion-filled gelatine gel. The oil droplets are probably expelled during friction measurements, which is supported by literature, in which it was shown that gelatine gels could release oil droplets from emulsion-filled gels due to shear processes (Sala et al., 2007). As gelatine has a very high water holding capacity, serum release is less likely (Martin et al., 2016). Although not significant, gels containing starch showed a slight trend for higher friction forces $F_F$ than unfilled gels. This may be due to the starch granules providing a more rough or sticky surface or the additional binding of free water by starch granules. Our setup is, thus, capable of distinguishing friction behaviour of gels differing in composition. Besides the properties of the continuous phase, also the dispersed phase affects the friction behaviour.
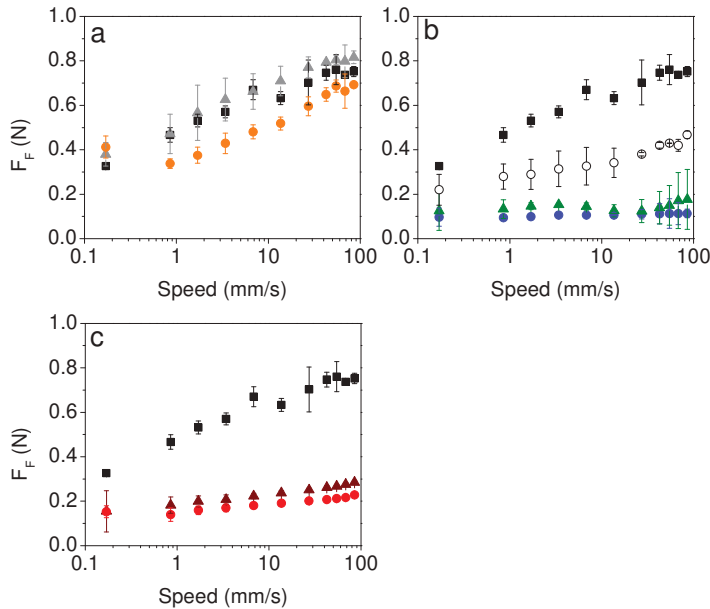
**Figure 2. Friction force ($F_F$) between soft solid gelatine gels against PDMS probe, as a function of speed. a) Gelatine gels differing in composition/filler, without added lubricant. Black squares: gelatine gel, orange circles: emulsion-filled gelatine gel; grey triangles: starch-filled gelatine gel ($F_{sample}$:14.94, p<0.001; $F_{speed}$: 72.48, p<0.001). b) Gelatine gel, without filler with various added lubricants (on the surface). Black squares: no lubricant, blue circles: water, green triangles: saliva, open circles: oil ($F_{sample}$:83.81, p<0.001; $F_{speed}$: 4.28, p<0.001). c) Gelatine gel boli. Black squares: (intact) gelatine gels. Red circles: 8 mm particles; dark red triangles: 4.5 mm particles ($F_{sample}$:83.97, p<0.001; $F_{speed}$: 9.34, p<0.001). The average from 3 measurements is shown with error bars representing a 95% confidence interval.**

Water, human saliva, and oil were individually added as lubricants on the surface of the gelatine gels without fillers to investigate the effect of liquids on the gel surface on friction behaviour (Table 1). As expected, the friction force $F_F$ decreased significantly (p<0.05) upon addition of water, saliva and oil (Figure 2b), indicating that all tested liquids lubricated the surface of the gelatine gels to some degree: samples lubricated with water or saliva showed the highest decrease, to 0.1 N, but showed no significant difference (p<0.05) between each other. The addition of the lubricants decreased the adhesive forces. Oil decreased friction slightly less than the aqueous lubricants to a friction force of 0.2-0.4 N (p<0.05). The reason for the observed differences in friction behaviour lies in the surface wettability of the gel. Gelatine is a highly hydrophilic matrix and therefore has a high affinity for hydrophilic lubricants, such as water and saliva. Hydrophobic oil cannot wet the surface as efficiently as the water-based lubricants; thus, friction forces are higher. When these lubricants were added on top of the surface of emulsion-filled gelatine gels or starch-filled gelatine gels, similar trends were obtained (data not shown).

Upon producing an artificial bolus and changing the contact type of the tribological setup from a PDMS/gel to a PDMS/bolus contact (Figure 2c), a substantial decrease in friction force is observed. However, the observed friction force did not differ significantly (p>0.05) between model boli with a particle size of 4.5

and 8 mm. While the food is broken down into smaller bolus particles, the apparent contact area between the bolus particles and the upper probe decreases. In consequence, the probe is now not in contact anymore with the sample over the entire area. Therefore, adhesion between the probe and the bolus particles is reduced, as compared to the intact gel, and thus the friction force was observed to decrease ($p<0.05$). It should also be considered that the bolus has to be considered as a deformable viscoelastic material. The friction force is therefore not only depending on the surface properties, but is also influenced by particle-particle interactions, deformation of the gel particles, and energy dissipation during movement (Rigney & Hirth, 1979).

The friction forces between the rough PDMS and (i) the intact sausages and (ii) simulated sausage boli, varying in bolus particle size (4.5 mm, Figure 3a; 8 mm, Figure 3b) and amount of lubricant (saliva) are shown in Figure 3. Intact solid sausages with added saliva led to overall higher friction forces $F_F$ compared to the gelatine gels with added saliva. This phenomenon can be related to the fact that gelatine gels generally have a very smooth surface (Grover et al., 2012), while the surface of sausages might be less smooth. By decreasing the sausage to a bolus with bolus particle sizes of 8 mm (Figure 3b), the friction force $F_F$ between the probe and the bolus was reduced significantly ($p<0.05$). By increasing the saliva content, the friction Force $F_F$ further decreased. When the bolus particle size was reduced even more (4.5 mm, Figure 3a), we can see a significant ($p<0.05$) influence of saliva concentration on the friction behaviour. In contrast to boli with large particles, saliva addition in small quantities (up to 20% saliva) increased friction forces $F_F$. Larger quantities of saliva were required to reduce the friction force $F_F$ between the probe and the bolus than for the intact sausage. We suggest that especially with the small-sized sausage bolus particles, adhesion between the particles and the probe is of relevance, where saliva is used to hold the particles together in a bolus. The higher friction force for the small particles might relate to the larger total surface area of the small particles, compared to that of larger particles; thus, more adhesion with the upper probe could take place. The adhesion also increases between the food boli particles within the bolus as saliva increases the cohesion and stickiness of the bolus, thereby making it more difficult for the probe to move. These two factors lead to an increase in the friction force. When increasing saliva content even further, friction decreases, as cohesiveness decreases. Another contributing factor might be slip phenomena (Davies & Stokes, 2008). This finding confirms that saliva aids the lubrication of the boli by decreasing adhesion, at sufficient amounts of saliva, thus facilitating swallowing. A comparable observation of such a critical moisture content on the effect of cohesiveness of particulated solids was also shown by research using a ring shear tester (Tobin et al. 2017).

In terms of bolus particle size, we suggest that with an increased surface area, more saliva is used to stick the particles together within the bolus, and less saliva is available to provide lubrication between the bolus and the probe. Thus, more saliva is required to facilitate lubrication. Overall, we can see that the friction behaviour of sausages is (particle) size and lubricant concentration-dependent.
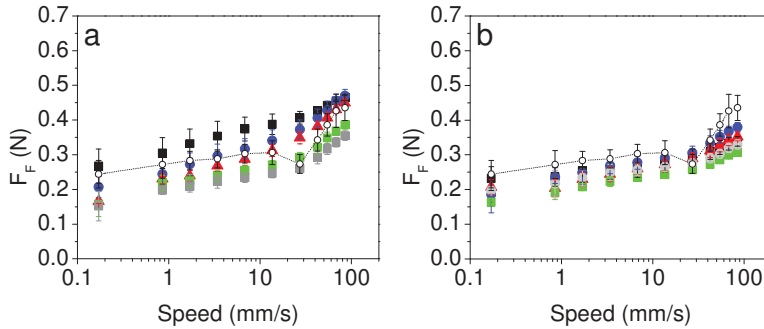
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**Figure 3. Friction force ($F_F$) between a rough PDMS probe and a sausage bolus of a) 4.5 mm particles ($F_{sample}$:23.82, p<0.001; $F_{speed}$: 66.72, p<0.001), and b) 8 mm particles, against a PDMS probe with saliva as lubricant ($F_{sample}$:17.8, p<0.001; $F_{speed}$: 66.36, p<0.001). Saliva content varied between 5% (black square), 10% (blue circle), 20% (red triangle), 30% (green square) and 40% (grey square). White circles with the dotted line indicate intact sausage (2.5 mL saliva). The dotted line has been added to guide the eye. Average values were obtained from 3 measurements with error bars indicating a 95% confidence interval.**

These results show that differences in food composition, lubricant content and boli particle size affect the measured friction behaviour of intact soft solid foods and food boli and that the presented set-up can measure these differences in friction behaviour. Compared to other tribological setups, the strength of this setup lies in its versatility. Many intact soft solid foods can be measured, and this method allows to measure the friction behaviour between a probe and soft solid foods directly. In many other tribological setups, the food often must be present as small particles between two measuring surfaces. Like all other setups, a limitation of this methodology is that the friction behaviour is system dependent, which means that the frictional behaviour obtained depends on parameters such as the surface properties of the probe. In a next step, it would be of interest to test and relate the findings made with this setup to results obtained from a sensory study, to provide insights in how the friction behaviour of soft solid foods relate to sensory perception.

## 8.4 Conclusions

In this short communication, we present a tribological setup to quantify tribological behaviour of intact soft solid foods and food boli. The setup consists of a PDMS probe, applying a defined normal force directly onto soft solid foods. The food is moved in a reciprocal linear motion against the probe and the resulting friction force $F_F$ is quantified as a function of speed. Differences in the composition of the food were shown to change the friction behaviour of soft solid foods considerably; present oil droplets in gelatine gels were seen to decrease friction. For food boli, the addition of saliva, as expected, significantly reduced friction, but the amount of saliva required was dependent on the particle size of the boli fragments. We believe that further research in tribology, with continuously improving measurement setups, is valuable to gain more insight into oral processing.

# Chapter 9 - General Discussion

## Introduction

This thesis aimed to study the effect of spatial oil droplet inhomogeneity in emulsions and emulsion-filled gels on rheological properties, tribology and sensory characteristics. In Figure 1, a schematic representation is provided of how the knowledge gained in this thesis fits with the current literature. The focus of the thesis was on dispersed oil droplet properties and droplet cluster properties (cluster size, strength and type).

In this chapter, first, the main results of the thesis are summarised. Then, implications of oil droplet clustering on the rheological, tribological and sensorial properties are discussed, considering possible applications. Subsequently, a case study, based on findings of this thesis, is provided (**Chapter 7**). Finally, limitations of the current studies are discussed, and recommendations for future work are provided.
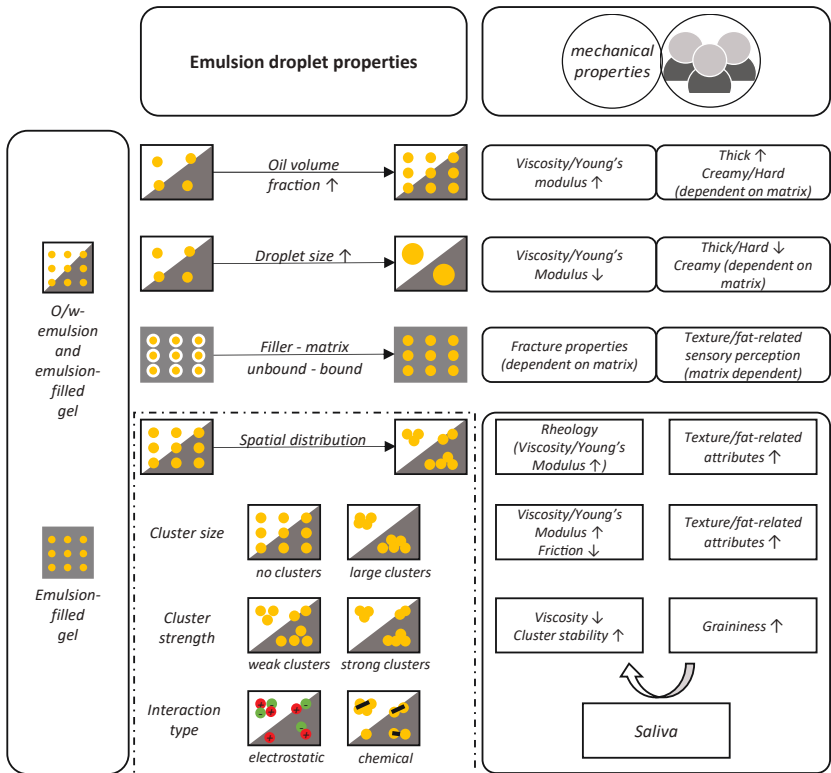


**Figure 1. Linking the microstructure to the physical food properties and sensory perception. ↑ indicates an increase, ↓ indicates a decrease. Half-filled squares refer to both emulsions and emulsion-filled gels, filled squares refer to emulsion-filled gels only.**

## Main results of the thesis

An overview of the main effects of oil droplet inhomogeneity on rheological and sensory properties of emulsions and emulsion-filled gels is provided in Table 1. The oil droplet distribution in oil-in-water (o/w) emulsions can be controlled for droplet cluster size and droplet interaction strength. By using chemical crosslinking of protein-stabilised droplets with polyphenols or electrostatic clustering of droplets with opposite surface charge ("hetero-aggregation"), controlled oil droplet clustering was achieved. In o/w emulsions, clustering led to an increase in viscosity and shear-thinning behaviour (**Chapter 2**). When the oil volume fraction of a clustered emulsion is increased, a soft glassy system or an emulsion gel is already formed at φ > 0.3, so at volume fractions well below maximum packing fraction of random close packing, φ ≈ 0.64 (**Chapter 7**). Creaminess and thickness intensities of clustered o/w emulsions (φ = 0.2) were significantly higher than those of non-clustered o/w emulsions with the same oil content (**Chapter 3**). In emulsion-filled gels, clustering of the oil phase was achieved on μm- and mm-length scale. The Young's modulus of emulsion-filled gels increased by 25% upon μm-scale clustering. Millimetre-scale clustering showed no effect on rheological properties of emulsion-filled gels. However, redistributing the oil might have influenced the rheological properties of the continuous gel phase, due to a local fat-reduction or fat-increase. Texture-related sensory attributes (hardness and heterogeneity) were influenced more by μm-clustering, while fat-related attributes (*e.g.* coating) were influenced stronger by mm-scale clustering (**Chapter 4**). Strong, chemically crosslinked oil droplet clusters increased thickness and grainy perception of emulsions with increasing cluster size. In emulsion-filled gels, incorporation of oil droplet clusters led to an increase in modulus and a decrease in fracture stress and strain. Emulsion-filled gels with strongly interacting, cross-linked clusters were perceived as harder and grainier compared to gels with weakly interacting, hetero-aggregated oil droplet clusters (**Chapter 5**). The results obtained with model emulsion-filled biopolymer gels were translated into a processed protein-based particle gel. **Chapter 6** demonstrated that clustering of oil droplets is a strategy that allows for fat-reduction in such a system while maintaining rheological and sensory properties. To study tribological properties of food boli, an experimental methodology from research on hydrogels was adopted to measure friction properties of intact solid foods and food boli (**Chapter 8**).

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**Table 1. Overview of the effects of oil droplet clustering on rheological properties and sensory perception. WPI: whey protein isolate, DATEM: diacetyl tartaric acid esters of monoglycerides, GSE: grape seed extract (source of polyphenols), φeff: effective oil volume fraction, Fract.: Fracture. ↑ refers to increase, ↓ refers to a decrease, ↘/↗ refer to a trend in de- or increase, an increasing number of arrows indicates a very strong effect.**

| Clustering mechanism | | | Oil droplet clusters with weak interactions | | Oil droplet clusters with strong interactions | - |
|---|---|---|---|---|---|---|
| | | | *weak electrostatic* | *strong electrostatic* | *physicochemical* | *"Gel-in-Gel"* |
| Emulsifier | | | *Gelatine-WPI* | *Gelatine-DATEM* | *WPI-GSE* | *WPI* |
| Cluster size (μm) | | | 1-50 | 1-50 | 1-150 | mm-range |
| Cluster strength | | | ↑ | ↑↑ | ↑↑↑ | → |
| Mech. properties | Emulsion | *Viscosity* | ↑ (cluster size) | ↑ (cluster size) | ↑ (cluster size) | - |
| | | *Friction* | ↓ | ↘ | small ↓, medium/large ↑ | - |
| | | *φ_eff* | ↑ | ↑ | ↗ | - |
| | Emulsion-filled gel | *Modulus* | too weak to be incorporated | ↑ | ↗ | → |
| | | *Fract.stress* | | ↘ (emulsifier x matrix) | ↘ | ↓ |
| | | *Fract.strain* | | ↘ | ↘ | ↘ |
| Sensory perception | Emulsion | | ↑ creamy, thick, coating        ↓ watery | ↑ thick, fatty, sticky   ↗ grainy   ↓ watery | ↑ creamy (large clusters), thick   ↗ grainy   ↓ watery | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Emulsion-filled gel** | - | ↑ hard | no effect on fat-related attributes | ↑ hard, grainy | ↗ creamy (modulus corrected) | ↓ melting | ↑ coating | ↓ wateriness (if ↓ fat in outer phase) |

## Rheology and microstructure

### Rheology of clustered o/w emulsions

Oil droplet clustering increases the viscosity and introduces shear-thinning behaviour (**Chapters 2,3,5**) below an oil volume fraction ≈ 0.3. At oil volume fractions above ≈ 0.3, clustering can lead to emulsions displaying a predominantly elastic character (**Chapter 8**). The increase in viscosity and the induction of elasticity depend on (i) the crowding of the droplets and the entrapment of the continuous phase, thus, an increase of the effective oil volume fraction, (ii) the creation of non-spherical droplet clusters (Dickinson & Golding, 1997; Genovese, 2012; Tadros, 1996) and (iii) the type and strength of the droplet-droplet interactions. Similar mechanisms have been suggested for different colloidal systems, including silica dispersions and protein solutions, showing that clustering strongly affects the rheology of dispersed phase systems (Chen, Øye, & Sjöblom, 2007; Purwanti et al., 2011).

Clusters with stronger attractive electrostatic interactions displayed higher viscosities than clusters bound by weaker interactions. The reason was that at a given shear rate, weakly interacting emulsion clusters have a smaller equilibrium size than strongly interacting ones (Genovese, 2012). Therefore, the effective volume fraction and viscosity of clusters decreases for weakly interacting clusters more than for strongly interacting clusters. With further increasing cluster strength (GSE-based clusters), however, a lower viscosity was observed compared to equally sized electrostatically stabilised clusters. This effect is not derived from lower stability, but a denser packing of the clusters. The clusters with strong interactions have a very dense structure. Therefore, the entrapment of the continuous aqueous phase is lower than for hetero-aggregated clusters. Thus, when attractive interactions between oil droplets are very strong, the dense structure of the formed clusters hinders the entrapment of water, and the effect of clustering on viscosity is reduced. As soon as emulsions can form a space spanning network, emulsions with higher droplet-droplet interaction strength show a higher elastic modulus than electrostatically stabilised ones.

Our findings showed that there seemed to be an optimum between shear stability and packing density to obtain the largest increase in viscosity. Overall, to apply clustering of oil droplets to structure foods in the context of fat reduction and to increase viscosity, one should aim for creating clusters of intermediate cluster strength with high capability to entrap water.

### Rheological properties of double and clustered emulsions

The crowding of the dispersed phase can also be found in double emulsions (Matos, Gutiérrez, Martínez-Rey, Iglesias, & Pazos, 2018; Oppermann, Verkaaik, et al., 2017; Pal, 2011). In w/o/w emulsions, the oil droplets present in the o/w emulsion contain dispersed water droplets. Even though these emulsions are typically not used to modulate the rheology of an oil-in-water mixture, the crowding affects the rheology of double emulsions. The properties determining the rheological behaviour of double emulsions show surprising similarities with clustered o/w emulsions.

The flow behaviour of double emulsions depends on four factors: (i) the volume fraction of droplets within the dispersed droplets, $\varphi_{PE}$, (ii) the volume fraction of the dispersed phase, $\varphi_{ME}$, (iii) the ratio between the

viscosities of the continuous phases of the inner emulsion and the double emulsion K, and (iv) the ratio between the internal droplet viscosity and the continuous phase of the inner emulsion $K_2$. Thus, the viscosity of the double emulsions $\eta_{ME}$ can be expressed as (Pal, 2008):

$$\frac{\eta_{ME}}{\eta_1} = 1 + Z_2 * \varphi_{ME}$$
1

where $\eta_1$ is the viscosity of the continuous outer phase and $Z_2$ is a crowding factor that includes the ratio of continuous phase viscosity to the viscosity of the double-emulsion matrix ($K_{21}$), the ratio the viscosity of the inner droplets to the continuous phase viscosity ($K_{32}$).

For clustered emulsions, the viscosity is determined by the effective oil volume fraction, $\varphi_{eff}$, which depends on the actual oil volume fraction and the interaction strength of the clusters. For clustered emulsions, the viscosity, $\eta_{emulsion}$, at oil volume fractions higher than 0.01 can be estimated by the Krieger-Dougherty equation (Equation 1, in **Chapter 2**).

The relative viscosity of double and clustered emulsions as a function of $\varphi_{ME}$ for double emulsions and as a function of $\varphi_{eff}$-$\varphi_0$ for clustered emulsions are given in Figure 2. Crowding of oil droplets affects the viscosity of both emulsions, independently of the crowding strategy. However, even though both types of emulsions increase the viscosity, clustering by attractive interactions increases relative viscosity more than double emulsions.
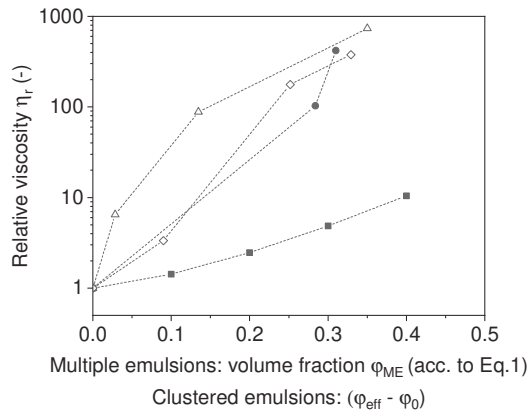


**Figure 2. Relative viscosity of clustered/multiple emulsions vs $\varphi_{ME}$ (multiple emulsions)/increase in eff. viscosity ($\varphi_{eff}$- $\varphi_0$) (clustered emulsions). Emulsion symbols: Triangles: weak electrostatically clustered emulsions (GW, see Chapter 2), Diamond: strong electrostatically clustered emulsions (GD, see Chapter 2), black circle: strongly cross-linked emulsions (GSE, see Chapter 2), black squares: calculated relative viscosity for w/o/w emulsions, based on Eq.1., We assumed: sunflower oil (48.8 mPa·s (Diamante & Lan, 2014)), water (1 mPa·s), oil volume fraction of the primary emulsion $\varphi_{PE}$ of 0.2 (similar to the shown clustered emulsions). Dotted lines are added to guide the reader.**

Rheological properties of emulsion-filled gels

Clustering of oil droplets in an emulsion-filled gel affects elastic modulus due to an increasing effective oil volume fraction of the dispersed phase (**Chapters 4,5**). Relevant factors are the modulus of the clusters,

the modulus of the matrix, the interaction between matrix and cluster, and the shape of the clusters. With increasing interaction strength between the droplets in the clusters, clusters become increasingly stiff, which leads to a larger increase of the modulus compared to weakly interacting clusters. This means that the hetero-aggregates, which are less stiff than strongly bound clusters (GSE-based), increase the gel stiffness less than strongly interacting clusters. Clustering of oil droplets affects the modulus of a gel, depending on the matrix modulus. As described in literature, the reinforcement of a matrix by fillers depends on the ratio between the stiffness of the filler and that of the matrix (Oliver, Berndsen, van Aken, & Scholten, 2015b). Thus, the effects of clustering on gel stiffness may be larger in less stiff matrices. Also, the interaction between clusters and matrix matter, as matrix reinforcement depends on the interaction between filler and matrix (Sala, van Aken, Stuart, & van de Velde, 2007a). While clusters that interact with the matrix can lead to reinforcement, inactive or unbound clusters might lead to matrix weakening. We observed for hetero-aggregated clusters, which consist of negatively- and positively charged droplets, less matrix reinforcement then for stiff clusters. Next to differences in cluster stiffness, the electrostatically bound clusters were less stable compared to the strongly bound clusters. Therefore, the overall effect on the material reinforcement was lower compared to the strong (GSE-type) clusters.

Clustering of oil droplets, and in specific cluster strength, affected fracture properties of emulsion filled gels (Figure 3). Strongly bound clusters (**Chapter 5**) can be used as hard fillers to increase the brittleness of the gels; larger clusters lead to more brittle gels. For clusters with more weakly interacting droplets, the fracture properties are more influenced by the droplet-matrix interactions. Such changes in behaviour have also been seen in the case of bound droplets in polymer gels (Sala, van Vliet, Cohen Stuart, Aken, et al., 2009).
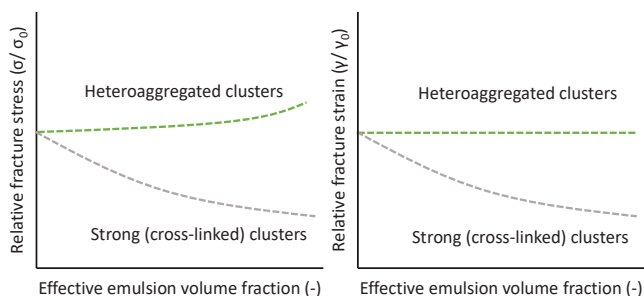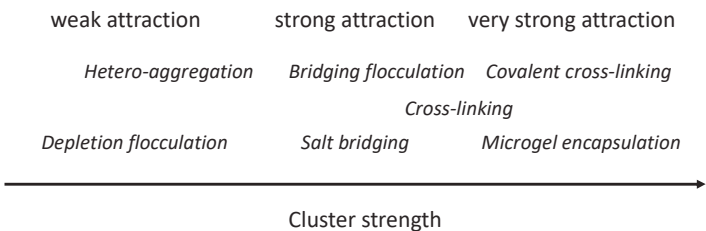


**Figure 3. Effect of emulsion droplet clustering on fracture stress and strain of polymer gels.**

Controlling cluster size and strength

Cluster size and strength strongly affected material properties. For electrostatic interactions, the cluster strength could be varied by changing the charge difference between two oppositely charged emulsifiers and the pH; larger charge differences lead to stronger interacting oil droplets in clusters. By varying the ratio between positively and negatively charged droplets, cluster size can be changed. More strongly interacting clusters were obtained by clustering negatively-charged droplets with divalent cations, and the strongest interactions were obtained through crosslinking of oil droplets with polyphenols. By increasing the concentration of cross-linker, clusters of various size could be obtained. Due to the critical role of the

cluster parameters, it is interesting to contemplate how alternative clustering approaches might perform in modifying the rheological properties of emulsions or emulsion-filled gels. Some of these approaches are shown in Figure 4 and compared in terms of cluster strength. An alternative approach to obtain weak attractive interactions is depletion flocculation. By incorporation of large polymers, depletion flocculation and indirect attractive droplet-droplet interactions can be obtained. The main limitation is that such systems require high volume fractions of dispersed colloids and would not easily allow to control cluster size (Datta et al., 2011). Therefore, clustering by electrostatic interactions is advantageous. Alternative approaches to obtain stronger clusters include bridging flocculation with biopolymers (Cao, Dickinson, & Wedlock, 1990) or salts (Jansson, Eriksson, & Skagerlind, 1991). Even stronger clusters could be obtained by covalently linking droplets, *e.g.* by enzymes (Christiane Maier et al., 2015) or encapsulating droplets into microgel particles (Feng, Yue, Wusigale, Ni, & Liang, 2018), which could be seen as an alternative strategy to obtain mm-scale clustering. Clusters obtained by electrostatic attraction or crosslinking with polyphenols provided the best control over cluster size and cluster strength in the context of this research.

| weak attraction | strong attraction | very strong attraction |
|---|---|---|
| *Hetero-aggregation* | *Bridging flocculation* | *Covalent cross-linking* |
| | *Cross-linking* | |
| *Depletion flocculation* | *Salt bridging* | *Microgel encapsulation* |

Cluster strength

**Figure 4. Schematic summary of approaches to obtain attractive interactions between emulsion droplets.**

## Tribology

The distribution of oil droplets influences the lubrication properties through (i) the increase in effective oil volume fraction, (ii) the cluster size and (iii) the droplet-droplet interactions. These factors could be used to tune the lubricity of foods.

The effect of increasing the volume fraction of the dispersed phase on lubricity seems to be quite general (Anvari & Joyner (Melito), 2017; Kimura & Okada, 1989). Aqueous dispersions of oil droplets, whey protein particles or glass particles have been shown better lubrication with increasing particle volume fraction. Two mechanisms are relevant for particle-based lubrication. Solid particles lubricate via a ball bearing mechanism, while for soft, deformable particles, the lubrication can be explained by the plate-out-theory. In the former case, the particles need to roll. Thus, spherical particles are required for ball bearing. For soft particles or droplets, the droplets need to deform and form a film. For both mechanisms, the main reason for the improved lubrication with increasing phase volume fraction seems the better separation between the tribo-pairs (Sarkar, Kanti, Gulotta, Murray, & Zhang, 2017; Yakubov, Branfield, Bongaerts, & Stokes, 2015). In this context, it was observed that an increase in effective volume fraction, similarly to an increase in real volume fraction, improved lubrication of clustered emulsions.

Also, the size of the clusters influences the lubrication behaviour of the emulsions. Both large weakly interacting and small strongly interacting oil droplet clusters are better lubricants than the non-clustered emulsions and large, strong clusters (GSE). To reduce friction, particles or droplet clusters should not be

too large so that they no longer can enter the gap between the tribo-pair. For the strongly clustered emulsions, it was observed that a cluster size of 70 μm and larger led to an increase in friction coefficient. Finally, the interactions among droplets can tune the ability of an emulsion to lubricate surfaces. Strong and dense (GSE-based) clusters also improve the lubrication, but only up to a size of 20 μm. When the cluster size of strong clusters becomes too large, the friction increases due to particles not being able to enter the gap or jamming of the particle clusters. A similar increase in friction has been observed with dispersions of particles and hydrophilised PDMS above a critical particle volume fraction of random close packing (Sarkar, Kanti, et al., 2017). These results show that to obtain good lubrication, one should aim to prepare large and soft clusters.

### Limitations of the tribo-pairs

Tribological studies are aimed to study oral processing and are typically performed with tribo-pairs consisting of PDMS and glass or PDMS and PDMS. PDMS is among the most popular materials to simulate the oral cavity (Bongaerts, Rossetti, & Stokes, 2007; Dresselhuis, de Hoog, Cohen Stuart, & van Aken, 2008; Sarkar, Andablo-Reyes, Bryant, Dowson, & Neville, 2019). Neither PDSM nor glass can entirely replace a tongue, and one might argue that biological tissue samples, such as pig tongues (Dresselhuis et al., 2008), would better reflect the properties of the human tongue. However, biological tissues have certain disadvantages compared to PDMS. In terms of availability, pig tongues are not always easily obtainable and quickly perish. Also, biological tissues show high inter-animal variability, and experiments carried out with these materials thus suffer from poor repeatability. Therefore, PDMS is currently still the material of choice. Its rheological and surface properties and its shape can be easily modified, and its frequent use allows to compare results among different studies.

Further optimisations of the material and surface properties, including a softer PDMS and PDMS with a defined roughness, might help to link tribological findings better to oral processing. In future studies, it would be beneficial to standardise the oral model materials and protocols used in tribological food research to compare results across different studies.

### Using tribology to study solid foods

Most research on the tribological properties of foods focuses on liquid or pourable semi-solids, such as custards, wine or yoghurt (Brossard, Cai, Osorio, Bordeu, & Chen, 2016; Dickinson, 2018; Morell, Chen, & Fiszman, 2017; Zhu et al., 2019). Much less research can be found for (semi-)solid foods. In this research, the tribology of solid foods was studied by first fragmenting them (Krop, Hetherington, Holmes, Miquel, & Sarkar, 2019), as also described in a previously reported method (Liu et al., 2015). In short, emulsion-filled gels were extruded through a syringe, surrogating the fragmentation occurring during oral processing. The obtained particles were used as a lubricant between the two surfaces of the tribometer. The results showed that the presence of oil droplets in an emulsion-filled gel changes the lubrication properties. We found that changes in lubrication were mainly related to changes in the rheological properties of the gels. In line with literature, also oil release and the interaction between droplets and matrix might affect the lubricity of fragments of solid foods (Liu et al., 2015). The fragmented gels can be seen as models for food boli. However, limited knowledge is available about the lubrication between intact food and oral surfaces. Therefore, a methodology was developed that allows measuring intact gels and food boli in contact with a solid (PDMS) surface (**Chapter 6**). To do this, inspiration was taken from tribological studies on hydrogels,

which are typically performed on intact gel matrices (Gong, 2006). We could show that the lubrication properties of both intact model gels and sausages could be determined and differed depending on structure and composition. We could point out that by including oil droplets in a gelatine gel, the friction of an intact gel against a PDMS surface decreases.

Furthermore, it was possible to differentiate between boli with different liquid content, by means of friction forces. This setup allows to gain sensible information about how food ingredients and food structure affect the (surface) friction of foods, how saliva can modify this friction and how boli are affected in their (surface) friction by composition and structure. Nonetheless, more research is necessary, including sensory studies and surface visualisations, to validate the applicability of this approach.

### Linking sensory perception and tribology

Tribology has been claimed to be a suitable tool for explaining fat-related sensory perception. However, to this point, only a few relations could be confirmed experimentally. These include correlations between the occurrence of high friction coefficients and astringency, as well as the link between low friction coefficients and a smooth/creamy sensory perception (Sarkar & Krop, 2019). In our studies, friction coefficients at speeds between 10 and 80 mm/s, typically in the mixed regime, were found to be negatively correlated to the creaminess. This finding is in line with literature, further confirming the link between lubricity and creaminess (Sarkar & Krop, 2019). We also observed that the slope of the mixed regime was correlated to fattiness/oiliness. This slope is related to how easily the emulsion reduces friction as a function of speed. If an emulsion easily separates and lubricates a tribo-pair, the Stribeck curve might show a steep slope in the mixed regime. Such a sample might also be perceived as oilier. However, to confirm the existence of a causal relation, further studies would have to be conducted.
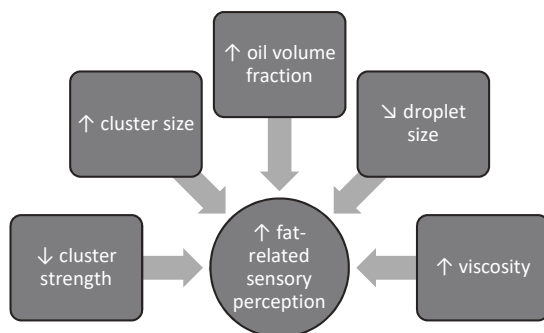
### Sensory perception

Clustering of oil droplets allows to alter the texture- and fat-related sensory perception of o/w emulsions and emulsion-filled gels. By tuning cluster type, cluster size and cluster strength, specific sensory attributes can be altered. In the following, strategies to modify specific attributes are further elaborated.

### O/w Emulsions

By clustering the oil droplet phase in o/w emulsions, the thickness perception of o/w emulsions can be considerably increased, and wateriness reduced. We found that both attributes were linked to the cluster size (**Chapters 3 and 5**). The reason for this increase is mainly the increase in emulsion viscosity. Thus, similar effects on sensory perception might be obtained by the addition of thickeners (xanthan, **Chapter 3**) or by increasing the total oil volume fraction. However, clustering of oil droplets is an attractive solution for food systems, as neither additional ingredients need to be added to the emulsions, nor fat and energy content is increased, while the rheological and tribological properties can be tuned. In line with thickness perception, creaminess of the emulsions can be increased with increasing cluster size. However, one has to keep in mind that when cluster strength is high, undesired perception of graininess is introduced. Strongly cross-linked (**Chapter 5**) and strongly electrostatically bound clusters (**Chapter 3**) lead to grainy perception at cluster sizes of roughly 10 µm. Emulsions clustered by weak electrostatic interactions were not perceived as grainy by panel members, even not at a large cluster size of 50 µm. The perception of graininess is linked to the detectability of particles, which has been shown to depend on particle stiffness

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and size (Imai, Hatae, & Shimada, 1995). By using weakly interacting clusters, one creates a cluster structure that might either fall apart during oral processing or is so easily deformable that it is not perceived in the mouth.

Thus, to modify specifically the fat-related sensory perception of an emulsion, one has to take into account the already known factors such as viscosity, oil volume fraction (Dickinson, 2018) and droplet size (Lett et al., 2016), as well as cluster size and cluster strength of clustered oil droplets (Figure 5).



**Figure 5. Parameters influencing the fat-related sensory perception in o/w emulsions. ↓ refers to a decrease and ↑ refers to an increase in a parameter which positively influences fat-related sensory perception. ↘ refers to a trend indicating an inverse relationship between this parameter and fat-related sensory perception.**

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### Emulsion-filled gels

The effect of clustering on the sensory properties of gels is less pronounced than the one for emulsions. Clustering of the emulsion droplets in an emulsion-filled gel can modify texture-related attributes such as hardness and brittleness, in line with changes of the rheological properties.

Like for emulsions, the incorporation of strongly bound clusters (GSE, **Chapter 5**) in gels leads to the perception of graininess. Thus, it was concluded that, within a range of moduli ratios between particle and matrix, the matrix modulus seems to have overall a weaker influence on the perceptibility of particles than the particle modulus, in line with literature (Shewan et al., 2020). As such, if one aims to increase the perceived hardness, hetero-aggregates with a high ratio of bound droplets and large clusters are preferred, as graininess perception can be avoided.

Oil droplet clustering can also be used to modify the fat-related sensory perception of emulsion-filled gels. To do so, the overall length scale of clustering has to be kept in mind. In terms of the length scale, large scale-inhomogeneity can be used to tune the fat-related sensory perception, such as coating and lingering. These effects are likely linked to changes in rheological properties of the continuous matrix, due to oil droplet enrichment or oil droplet depletion. One could expect that the larger the heterogeneity, the larger the effect (Mosca et al., 2012). Small scale-clustering, on the other hand, was found to allow the tuning of creaminess perception. While in **Chapters 4+5** studies on model emulsion-filled gels are reported, in **Chapter 6** we demonstrated that in processed protein-based particle gels, a reduction in fat-content can be achieved through droplet clustering while maintaining the sensory characteristics and the rheological properties of a full-fat type system. As clustering reinforces the stiffness, clustering allows to "correct" for

a reduction in gelling agent concentration or fat content. This finding might suggest that clustering mainly affects sensory perception through the modification of the rheological properties of food.

## Applications of controlled oil droplet clustering

To achieve the desired effects of oil droplet clustering on rheology, tribology and sensory, one has to optimise cluster size, strength and interaction type. In short, more loose clusters typically have more advantages than very dense clusters. However, they are of limited stability. Thus, in order to use clustering as a tool, one should preferably use clusters that are stable enough to be incorporated into the matrix, but weak enough to either fall apart during oral processing or remain undetected due to their low stiffness. Optimisations concerning electrostatic clusters, for example, could include the use of alternative cationic emulsifiers to replace gelatine (*e.g.* lysozyme). However, with regards to foods, cationic surfactants are limited. Nonetheless, clustered emulsions can not only be used to tune the rheology, tribology and sensory perception, but also control the rate of coalescence in emulsions.

## Modifying lubrication properties of o/w-emulsions by inducible coalescence

The sensory properties of o/w emulsions are strongly linked to the dynamic changes of the emulsions while we consume them (Dickinson, 2018; Lett et al., 2016; Vingerhoeds et al., 2009). O/w emulsions, when being consumed, are mixed and diluted with saliva and swallowed after a short oral residence time (Chen, 2014; Chen, 2009). Studies have shown that the perception of o/w emulsions can be influenced by flocculation or coalescence of oil droplets in the mouth. Dresselhuis (2008) demonstrated that the coalescence of emulsion droplets in the mouth leads to enhanced fat-related attributes, such as creamy, fatty and oily taste (Dresselhuis, de Hoog, Cohen Stuart, Vingerhoeds, & van Aken, 2008). It is believed that the oil droplets can form an oil layer more easily after coalescence, as they more easily form patches that would decrease the friction forces. One strategy to obtain droplets coalesce in the mouth is to use emulsifiers that can be destabilised during oral processing, such as starch (Tesch, Gerhards, & Schubert, 2002). To do so, starch is chemically modified with a hydrophobic group (octenyl succinic acid, OSA), which leads to improved emulsifying properties. Upon contact with saliva, the starch at the oil-water interface is cleaved by amylase and loses its stabilising function; the emulsion coalesces. A prerequisite for fast coalescence is, next to an emulsifier with controllable stability, the proximity of two (or more) droplets. When oil droplets are already in close vicinity, coalescence can occur more quickly. Based on this prerequisite, it was hypothesised that clustering of starch-stabilised oil droplets could significantly facilitate coalescence and, consequently, improve lubrication and sensory perception, compared to non-clustered starch-stabilised emulsions. To test this hypothesis, starch was modified, to obtain emulsifying starches of (overall) positive or negative charge. Upon combining starch-stabilised emulsions with a positive and negative charge, hetero-aggregated clusters were obtained. An overview of emulsion properties can be found in Table 2.

**Table 2. Overview of physical characteristics of o/w emulsions with non-clustered droplets and with clustered droplets stabilised with modified starch ($\varphi$= 0.2).**

| Non-clustered emulsions (Emulsifier) | Droplet size [µm] | ζ-potential [mV] | Consistency [mPa·s$^n$] | Flow index [-] | Critical strain [%] |
|---|---|---|---|---|---|
| *Positively charged starch* | 8.7 ± 0.3 | +4.58 ± 0.33 | 10.9 ± 2.2 | 1 | - |
| *Negatively charged starch* | 4.0 ± 0.2 | -21.5 ± 1.39 | 9.5 ± 1.6 | 1 | - |
| Clustered emulsions (Emulsifier) | Cluster size [µm] | | Consistency [mPa·s$^n$] | Flow index [-] | Critical strain [%] |
| *Positive : negative starch 3:7* | 21.4 ± 0.9 | - | 91.2 ± 22.6 | 0.32 | 2.04 ± 0.71 |

| | | | | |
|---|---|---|---|---|
| *Positive : negative starch 5:5* | 34.5 ± 6.9 | 126.2 ± 10.3 | 0.39 | 3.08 ± 0.42 |
| *Positive : negative starch 7:3* | 47.0 ± 4.5 | 112.5 ± 22.1 | 0.39 | 1.07 ± 0.43 |

Cluster sizes between 21 and 47 µm were obtained, with the largest clusters at a ratio of 7:3 of positively-to-negatively charged emulsions. The sensitivity to coalescence was tested using tribology and microscopy, by mixing the emulsion with amylase-solutions (activities adjusted according to literature, (Minekus et al., 2014). Microscopy images show that no coalescence was obtained in emulsions stabilised with negatively-charged starch (Figure 6A and B), but emulsions stabilised with positively-charged starch did show considerable sensitivity to enzymatic breakdown (Figure 6C and D). These effects were also observed in tribology. In the case of negatively-charged emulsions, no change in the friction coefficient was seen (Figure 6E). The friction coefficients for positively-charged emulsions were observed to decrease (Figure 6F). We suggest that the negatively-charged starch might have a very high degree of substitution; thus, the enzyme might have been hindered in degrading the starch, as also suggested in other research (Viswanathan, 1999). Positively-charged starch might have a lesser degree of substitution. Additionally, even the electrostatic attraction between the positively-charged starch and the negatively-charged enzyme (Dubey, Suresh, Kavitha, Karanth, & Umesh-Kumar, 2000) could contribute to the improved enzymatic activity.
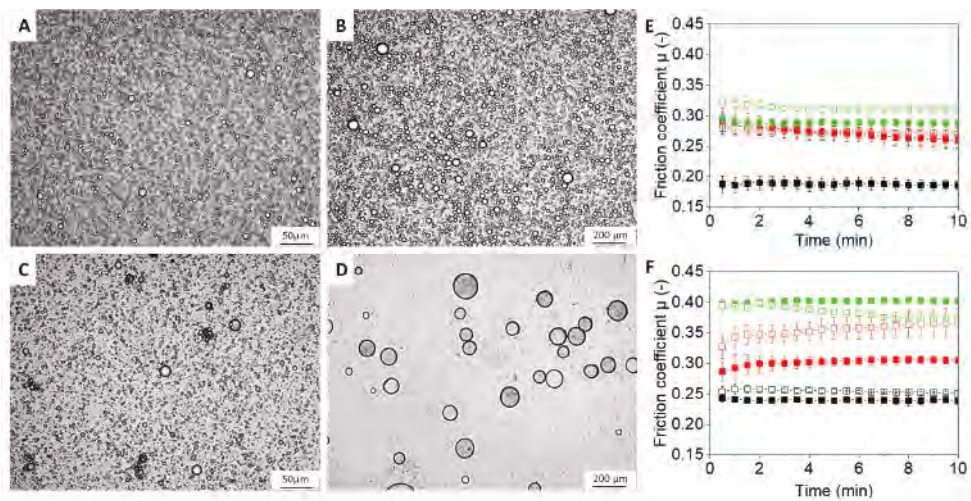
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**Figure 6. A-D: Micrographs of starch-stabilised emulsions – effect of amylase treatment. A+B: negatively-charged starch, C+D: positively charged starch. A+C: before incubation with amylase, B+D: after incubation (10 min) with amylase. E+F: Friction coefficients as a function of time of o/w emulsions (10% oil) stabilised with (E) negatively-charged starch or (F) positively-charged starch, during incubation with alpha-amylase. Filled symbols show the friction behaviour of emulsions without enzyme, and open symbols represent emulsions with amylase. Black lines/symbols represent a speed of 1 mm/s, red lines/symbols a speed of 10 mm/s and green lines/symbols a speed of 100 mm/s. Error bars indicate the standard deviation (n=3).**

Upon clustering of the emulsions, the rate and extent of coalescence of the emulsion droplets increased even further. The overall reduction in friction behaviour was faster and with a steeper slope compared to the non-clustered emulsions. (Figure 7A, B and C)
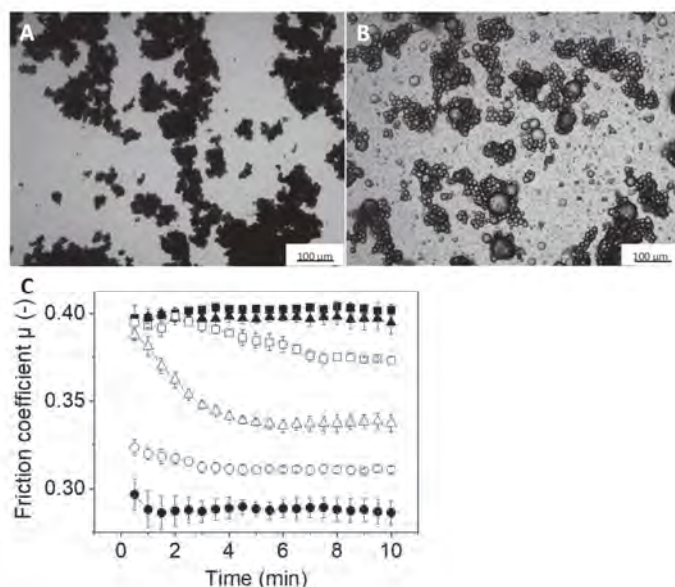
**Figure 7. A+B: Micrographs of an emulsion before tribological measurement (A) and after 4 min of tribological measurement (B). C: Friction coefficient of starch-stabilised emulsions over time of 10 min at a speed of 100 mm/s. Open symbols with enzyme, full symbols without enzyme (positive starch: squares; negative starch: circles; clustered starch (ratio 7:3): triangle).**

Based on these findings, it can be concluded that the controllable clustering of o/w emulsion might significantly affect the coalescence behaviour of oil droplets. We would expect that a facilitated coalescence between emulsion droplets would lead to a positive influence on perception. We could, however, not conclusively deduce this from the tribology experiments, as the overall friction coefficients of clustered emulsions were found to be higher than the emulsions stabilised by negatively-charged starch. Unfortunately, it was not possible to perform any sensory tests to confirm the hypothesis as the positively-charged starch is not commercially available as a food-grade ingredient, and therefore not suitable for sensory testing. To perform a sensory study, food-grade starches would be required, but are currently not commercially available. The findings might be used in foods where a fast coalescence during the residence time in the mouth can be desired.

## Obstacles in the application of clustered o/w emulsions

To obtain flocculated emulsion droplets is relatively straightforward, and flocculation might occur in many processes and formulations unintentionally. However, to use clustering of emulsion droplets purposefully, several obstacles in the application should be considered. These obstacles relate to two main factors: (a) the stability of the overall system and (b) the stability of the clusters. As clustering of oil droplets tendentially leads to an increase in creaming rate, the shelf stability of liquid emulsions would be reduced at low oil volume fractions compared to non-clustered emulsions (Figure 8). Potential strategies to counteract this phenomenon depend on the overall oil volume fraction of the system. At high oil volume fraction of about 30% oil or higher, one can increase clustering until a space-spanning network is obtained.

At these oil concentrations and above, a clustered emulsion forms a network, which yields upon deformation (**Chapter 8**). Upon clustering, the emulsion thus remains stable at oil volume fractions well below the maximum packing fraction (>0.64). In emulsions with oil volume fractions below 30%, one might either keep the cluster size low to limit the creaming or reduce creaming by addition of weighting agents (Chanamai & McClements, 2000).
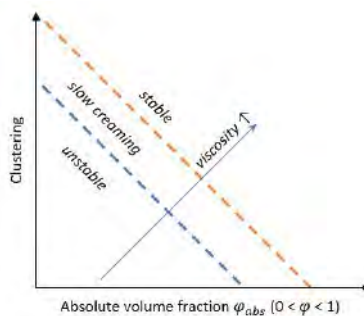


**Figure 8. Schematic representation of the interplay among volume fraction, clustering, stability and viscosity in emulsions with clustered oil droplets.**

The interaction type mainly limits the cluster stability of emulsion droplet clusters in o/w emulsions. Electrostatically clustered emulsion droplets might be hindered in the cluster (re)-formation at high ionic strengths due to screening of charges. Also, strong changes in pH might lead to a decrease of the charge of the emulsifiers and might be therefore detrimental for the clustered structure. For strongly bound clusters, *e.g.* GSE-clustered droplets (compare **Chapters 2 and 5**), limiting factors are shear forces. In emulsion-filled gels, the limiting factors are the cluster strength and the interactions between the droplets and the matrix. Clusters stabilised by weak electrostatic interactions were too weak to remain intact while being incorporated into a gel (low acyl gellan). Shear forces due to mixing led to complete disintegration of the oil droplet clusters. Thus, matrices with high viscosity should be avoided, or stronger interacting clusters should be used. However, clusters should be weak enough to avoid the perception of clusters as particles leading to graininess. Stronger clusters reduced slightly in size (by about 10-15%), but could be successfully introduced into gel matrices (low acyl gellan). Also, the gel matrix by itself can limit the successful incorporation of clusters. Specifically, for hetero-aggregated emulsions, a charged matrix might interfere in the structure and further reduce cluster size. To still incorporate hetero-aggregated clusters into such a matrix, fast gelation and low shear forces should be applied. The presence of di- or multivalent ions might destabilise hetero-aggregated clusters, and, at the same time, introduce oil droplet clusters stabilised by salt-bridges.

## Guidelines for applications and practical implications

Considering the practical implications of this thesis, several guidelines of potential practical application and implementation can be formulated. One of the key findings of this thesis is the substantial change of rheological/tribological properties and sensory perception of emulsions upon oil droplet clustering. The extent of the changes depends on the clustering methodology and the length scale of the inhomogeneity.

These insights could be implemented in the reformulation of products:

- Reduction of thickeners in foods. The clustering of droplets in emulsion increases the emulsion viscosity. This increase in viscosity reduces the need for the addition of thickeners. Therefore, thickeners could be partly removed from foods, such as high-fat dairy products. Droplet clustering introduces a strong shear thinning flow behaviour in emulsions, mimicking the behaviour of systems containing thickeners such as xanthan.

- Fast-changing rheological characteristics. The strong shear-thinning flow behaviour resulting from clustering could be beneficial for products targeted to specific consumer groups with limited swallowing capabilities. At rest, hetero-aggregated emulsions are viscous. During consumption, thus upon shearing, the hetero-aggregated emulsion clusters fall apart, and the viscosity is reduced.

- Fat reduction by clustering. The introduction of oil droplet clusters has been found to allow fat reduction while maintaining sensory perception (**Chapter 6**). Clustering could be used in various gel-like foods where the processing allows the incorporation of a clustered oil phase.

- Solid-like food texture without fermentation. Upon sufficiently high oil volume fraction (0.3-0.4) in the emulsions, clustering of oil droplets induces the formation of a space spanning network. Emulsions, which would be liquid if the droplets were homogeneously distributed, show upon clustering a semisolid-like consistency. This could be used for the development of new products, *e.g.* spreads.

## Further research

The presented work is mainly based on the possibility of clustering oil droplets via different types of interactions. We found that electrostatic attraction and crosslinking between droplets provide sufficient control over the process. However, more precise control over the interactions between droplets and their quantification would be desirable and could lead to an assembly of oil droplets into various structures, including chains or other supra-droplet morphologies, as shown for other types of colloids in nanotechnological bottom-up approaches (Bharti & Velev, 2015). One way of achieving such controlled oil droplet structures might be via the application of external electrical fields to emulsion droplets with ionic emulsifiers and the consequent stabilisation of such structures, by enzymatic processes or gelation. Not only would such systems provide fascinating rheological properties, but also materials with specific sensory properties might be achieved.

Moreover, it would be of interest to investigate how clusters of very strongly interacting droplets act as carriers of aqueous or non-aqueous substances; thus, whether clustering is suitable as a delivery approach. Another interesting question might relate to the digestibility of such clustered oil droplet structures. Assuming that clustered oil droplets limit the accessibility for lipase to the emulsion droplet, one might expect a reduced digestive rate of oil upon clustering. Such a manipulation of the digestive rate could have effects on the intake of the fat in foods. Finally, the current work has been performed on dispersed oil droplets in an aqueous surrounding. It is of interest how clustering of a dispersed aqueous phase affects a material with a continuous oil phase, such as margarine.

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# Summary

In this thesis, the structuring of the fat phase in o/w emulsions, in particular by modifying the spatial distribution of droplets and droplet-droplet interactions, was explored. It was investigated how the distribution and interactions might be used to tune rheological, tribological and sensory properties of foods.

To understand how the fat droplet distribution affects food, it is necessary to control the droplet distribution, for example, by clustering. We have, therefore, developed several approaches that lead to a controlled clustering of oil droplets in o/w emulsions. (**Chapter 2**) We tested a charge-based approach (hetero-aggregation), a chemical approach (clustering by polyphenols) and an enzymatic cross-linking approach. We have taken cluster properties, such as size and interaction strength into account. Droplet cluster size and droplet interaction strength could be varied in a controlled way by using chemical crosslinking (polyphenols) and charged-based clustering; enzymatic crosslinking did not result in the desired control. Large oil droplet clusters increased the effective oil volume fraction by up to 5x compared to non-clustered emulsions. With the increase in effective oil volume fraction, clustering increased the viscosity of emulsions by over 100x while keeping the actual oil content constant. Droplet-droplet interactions in emulsions can, therefore, be controlled, and by varying these interactions, the rheological properties of an emulsion can be changed.

We expected that clustering would also influence the sensory properties of an o/w emulsion. The second study (**Chapter 3**) investigated the effect of oil droplet cluster properties on the sensory properties of o/w emulsions. Creaminess and thickness intensities of clustered o/w emulsions were significantly higher than those of non-clustered o/w emulsions with the same oil content and comparable droplet size. With increasing cluster strength, graininess perception increased sharply. Thus, the oil droplet distribution and droplet-droplet interactions, considerably influence the sensory characteristics of an emulsion. As many foods are also emulsion-filled gels, strategies of clustering oil droplets in (soft-)solid systems were investigated in **Chapter 4**. To obtain inhomogeneities at different length-scales, we developed two approaches. One approach was based on hetero-aggregation (µm-scale) and one on a gel-in-gel methodology (mm-scale). Weak electrostatic interactions were not suitable for stabilisation of clustered oil droplets in emulsion-filled gels, as shear forces are too high to incorporate them and clusters disintegrate. For gels with stronger electrostatically-stabilised clusters, the Young's modulus increased by 25% compared to gels with non-clustered droplets. The fracture properties in these gels were hardly influenced by the clustering, but rather by matrix-droplet interactions. An inhomogeneous distribution of oil droplets at a mm-scale showed no effect on the total Young's modulus or fracture properties. For gels with droplet clustering on µm-scale, mainly texture-related attributes (hardness and heterogeneity) were influenced. For foods with mm-scale inhomogeneity, effects of inhomogeneity on oily perception and mouth-coating perception were found.

The incorporation of clusters often led to their disintegration due to high shear forces and interactions with other food components. Thus, in the fourth study (**Chapter 5**), the effects of strongly bound oil droplet clusters (by polyphenols) in gels and emulsions on sensory perception, rheology and lubrication behaviour were investigated. Strong clusters in o/w emulsions increased thickness perception. Cluster interactions

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were strong enough to keep clusters intact during oral processing. Due to this high cluster strength, grainy perception increased with increasing cluster size in the liquids. In gelled materials, the presence of clusters led to an increase in modulus and a decrease in both fracture stress and strain. Emulsion-filled gels with clusters were perceived as harder and grainy. We concluded that strongly bound oil droplet clusters act as quasi particles. Oil droplet clusters affect the rheological properties (**Chapter 2**) and sensory properties (**Chapter 5**), as a function of size and cluster strength.

In **Chapter 6**, we observed that clustering of oil droplets in a complex processed protein matrix could help to reinforce the matrix. The clustering of oil droplets could be a potential fat-reduction strategy in foods. In **Chapter 7**, we examined the effect of droplet-droplet interactions in emulsions with volume fractions above 0.3. In such emulsions, the droplet-droplet interactions strongly influence the rheology and yielding behaviour. By increasing the interaction strength between droplets, one can tilt the behaviour of a high volume fraction emulsion from a soft glassy state to a gel-like behaviour.

Linking sensory perception of emulsions-filled gels and friction properties is challenging as many tribological measurement approaches do not intend to measure the lubrication properties of intact foods (Gong, Iwasaki, Osada, Kurihara, & Hamai, 1999; Gong & Osada, 2002). Thus, in **Chapter 8**, a measuring setup using a polydimethylsiloxane (PDMS) surface in direct contact with an intact soft solid food/bolus was developed. The friction forces were measured when sliding the PDMS surface over the soft solid food. These depended strongly on the composition of the food. The presented methodology showed that we were able to quantify the effect of (i) composition and added fillers, (ii) serum or oil release and (iii) bolus particle size. We conclude that the friction behaviour of intact foods can be determined and might help in better linking sensory perception and material properties.

To conclude, oil droplets are important structuring agents in liquid and solid foods. By manipulating how the oil droplets are distributed in food and how they interact with each other in a food, it is possible to tune food properties, including rheology, tribology and sensory perception. By creating an inhomogeneous droplet distribution and introducing weak droplet-droplet interactions, an increase in desired food properties such as viscosity, lubricity and fat-related sensory perception can be achieved.

# Zusammenfassung

In dieser Arbeit wurde der Effekt der Fettphasenstrukturierung auf rheologische, tribologische und sensorische Eigenschaften von Öl-in-Wasser (Ö/W) Emulsionen und emulsionsgefüllten Gelen untersucht. Insbesondere wurden dabei die Modifizierung der räumlichen Verteilung von Öltröpfchen und Tröpfchen-Tröpfchen-Wechselwirkungen berücksichtig. Es wurde experimentell gezeigt, wie die Tröpfchenverteilung und deren Wechselwirkungen verwendet werden können, um physikalische und sensorische Eigenschaften von emulsionsartigen Lebensmitteln zu kontrollieren.

Um zu verstehen, wie sich die Fetttröpfchenverteilung auf Lebensmittel auswirkt, muss die Tröpfchenverteilung, beispielsweise durch Clusterbildung, steuerbar sein. Wir haben daher mehrere Ansätze entwickelt, die zu einer kontrollierten Clusterbildung von Öltröpfchen in Ö/W-Emulsionen führen (**Kapitel 2**). Wir haben einen ladungsbasierten Ansatz (Heteroaggregation), einen chemischen Ansatz (Clusterbildung durch Polyphenole) und einen enzymatischen Tröpfchenvernetzungsansatz getestet. Dabei wurden, im Speziellen, Clustereigenschaften wie Clustergröße und Interaktionsstärke berücksichtigt. Die Größe der Tröpfchencluster und die Stärke der Tröpfchenwechselwirkungen konnten mittels chemischer Vernetzung (Polyphenole) und Clusterbildung auf Ladungsbasis variiert werden. Die enzymatische Vernetzung führte nicht zu der gewünschten Prozesskontrolle. Große Öltröpfchencluster erhöhten den effektiven Ölvolumenanteil im Vergleich zu nicht geclusterten Emulsionen um das bis zu 5-fache. Mit der Zunahme des effektiven Ölvolumenanteils erhöhte das Clustering die Viskosität der Emulsionen um das über 100-fache, während der tatsächliche Ölgehalt konstant gehalten wurde. Tröpfchen-Tröpfchen-Wechselwirkungen in Emulsionen können daher gezielt eingesetzt werden, und durch Variieren dieser Wechselwirkungen können die rheologischen Eigenschaften einer Emulsion verändert werden.

Wir erwarteten, dass Clusterbildung auch die sensorischen Eigenschaften einer Ö/W-Emulsion beeinflussen würde. Die zweite Studie (**Kapitel 3**) untersuchte den Einfluss der Eigenschaften von Öltröpfchenclustern auf die sensorischen Eigenschaften von Ö/W-Emulsionen. Die Cremigkeit und (sensorische) Dickenintensität von geclusterten Ö/W-Emulsionen war signifikant höher als die von nicht geclusterten Ö/W-Emulsionen mit dem gleichen Ölgehalt und vergleichbarer Tröpfchengröße. Mit zunehmender Clusterstärke nahm die Wahrnehmung der Körnigkeit stark zu. Somit beeinflussen die Öltröpfchenverteilung und die Tröpfchen-Tröpfchen-Wechselwirkungen die sensorischen Eigenschaften einer Emulsion erheblich. Da es sich bei vielen Lebensmittel auch um emulsionsgefüllte Gele handelt, wurden in **Kapitel 4** Strategien zur Clusterbildung von Öltröpfchen in gelartigen Materialien untersucht. Um Tröpfcheninhomogenitäten (Tröpfchenclusterbildung) in verschiedenen Größenordnungen zu erhalten, haben wir zwei Ansätze entwickelt. Ein Ansatz basierte auf einer Heteroaggregation (µm-Maßstab) und einer auf einer Gel-in-Gel-Methode (mm-Maßstab). Schwache elektrostatische Wechselwirkungen zwischen Öltröpfchen waren nicht zur Stabilisierung von Öltröpfchenclustern in emulsionsgefüllten Gelen geeignet, da die Scherkräfte zu hoch waren und sich die Cluster destabilisierten. Bei Gelen mit elektrostatisch stabilisierten Clustern mit stärkerer Wechselwirkung erhöhte sich das Elastizitätsmodul des Gels um 25% im Vergleich zu Gelen mit nicht-geclusterten Tröpfchen. Die Brucheigenschaften in diesen Gelen wurden allerdings kaum durch die Clusterbildung beeinflusst, sondern

durch Matrix-Tröpfchen-Wechselwirkungen. Eine inhomogene Verteilung der Öltröpfchen (clustering) im mm-Maßstab zeigte keinen Einfluss auf das Elastizitätsmodul oder die Brucheigenschaften. Bei Gelen mit Tröpfchenclustern im μm-Maßstab wurden hauptsächlich texturbezogene sensorische Eigenschaften (Härte und Heterogenität) beeinflusst. Bei Lebensmitteln mit Tröpfcheninhomogenität im mm-Maßstab wurden Effekte der Inhomogenität auf die fettbezogene, sensorische Wahrnehmung festgestellt.

Der Einbau von Clustern führte aufgrund hoher Scherkräfte und Wechselwirkungen mit anderen Lebensmittelkomponenten häufig zu deren Zerfall. In der vierten Studie (**Kapitel 5**) wurden daher die Auswirkungen stark gebundener Öltröpfchencluster (durch Polyphenole) in Gelen und Emulsionen auf die sensorische Wahrnehmung, Rheologie und das Schmierverhalten untersucht. Starke Cluster in Ö/W-Emulsionen erhöhten die sensorische Wahrnehmung der Dicke. Die Cluster-Interaktionen waren stark genug, um die Cluster während der oralen Einnahme intakt zu halten. Aufgrund dieser hohen Clusterstärke nahm die Körnigkeitswahrnehmung mit zunehmender Clustergröße in den Emulsionen zu. In gelartigen Materialien führte das Vorhandensein von Clustern zu einer Erhöhung des Moduls und einer Verringerung sowohl der Bruchspannung als auch der Bruchdehnung. Emulsionsgefüllte Gele mit Clustern wurden als härter und körniger empfunden. Wir kamen zu dem Schluss, dass stark gebundene Öltröpfchencluster quasi als Partikel wirken. Öltröpfchencluster beeinflussen damit die rheologischen Eigenschaften (**Kapitel 2**) und sensorischen Eigenschaften (**Kapitel 5**) in Abhängigkeit von Größe und Clusterstärke. In **Kapitel 6** haben wir beobachtet, dass die Anhäufung von Öltröpfchen in einer proteinbasierten, prozessierten Gelmatrix zur Verstärkung der Matrix beitragen kann. Das Clustering von Öltröpfchen könnte eine potenzielle Strategie zur Fettreduzierung in Lebensmitteln sein. In **Kapitel 7** untersuchten wir den Effekt von Tröpfchen-Tröpfchen-Wechselwirkungen in Emulsionen mit Volumenanteilen über 0,3. In solchen Emulsionen beeinflussen die Tröpfchen-Tröpfchen-Wechselwirkungen die Rheologie und das Fließverhalten stark. Durch Erhöhen der Wechselwirkungsstärke zwischen Tröpfchen kann das Verhalten einer Emulsion mit hohem Ölvolumenanteil von einem weichen glasartigen Zustand zu einem gelartigen Verhalten gekippt werden.

Das Verknüpfen der sensorischen Wahrnehmung von emulsionsgefüllten Gelen und deren Reibungseigenschaften ist eine Herausforderung, da es an tribologischen Messansätzen zur Bestimmung der Schmiereigenschaften intakter Lebensmittel fehlt. Daher wurde in **Kapitel 8** ein Messaufbau unter Verwendung einer Polydimethylsiloxan (PDMS)-Oberfläche in direktem Kontakt mit einem intakten gelartigen Lebensmittel/Lebensmittelbolus entwickelt. Die Reibungskräfte wurden gemessen, wenn die PDMS-Oberfläche über das Lebensmittel geschoben wurde. Diese hingen stark von der Zusammensetzung des Lebensmittels ab. Die vorgestellte Methodik zeigte, dass wir den Effekt von (i) Zusammensetzung und zugesetzten Füllstoffen, (ii) Serum- oder Ölfreisetzung und (iii) Boluspartikelgröße quantifizieren konnten. Wir schließen daraus, dass das Reibungsverhalten intakter Lebensmittel bestimmt werden kann und dazu beitragen kann, die sensorische Wahrnehmung und die Materialeigenschaften besser miteinander zu verknüpfen.

Zusammenfassend lässt sich feststellen, dass Öltröpfchen wichtige Strukturgeber in flüssigen und festen Lebensmitteln sind. Durch Manipulieren der Öltröpfchenverteilung in Lebensmitteln und der Wechselwirkungen zwischen Tröpfchen und zwischen Tröpfchen und Matrix, ist es möglich, die Lebensmitteleigenschaften, einschließlich der Rheologie, Tribologie und der sensorischen Wahrnehmung,

zu steuern. Durch die Erzeugung einer inhomogenen Tröpfchenverteilung und dem Vorhandensein schwacher Tröpfchen-Tröpfchen-Wechselwirkungen kann eine Erhöhung gewünschter Lebensmitteleigenschaften wie Viskosität, Schmierfähigkeit und fettbezogener sensorischer Wahrnehmung erreicht werden.

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Philipp Fuhrmann, Wageningen 2020

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# About the author

Philipp Lawrence Rudolf Fuhrmann was born on the 8th of April 1991 in Vienna, Austria. After graduating from high school and working as paramedic, he started to study Food Science and Biotechnology in 2010 at the University of Natural Resources and Life Sciences Vienna (BOKU), Austria. For his bachelor thesis, he worked on the development of enzyme-based assays (ELISA) for the detection of food allergens. Thereafter, he continued his food journey with the master program Safety in the Food Chain at BOKU and an Erasmus semester (2014/2015) at Wageningen University, Netherlands. Philipp performed his master thesis at the cell biology department of Biomin, Tulln a.d. Donau, Austria, developing an *ex vivo* tissue culture to reduce the need for animal testing. His experiences at Wageningen University led him, after finishing his master's degree, back to the Netherlands, where he was appointed as a PhD candidate at TIFN and within the group of Physics and Physical Chemistry of Foods at Wageningen University in 2016. The work, presented in this thesis, was part of the project "Smooth bite for all".

He can be contacted by email: philippfuhrmann@hotmail.com

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# List of publications

## Peer-reviewed publications:

**Fuhrmann, P. L.**, Sala, G., Stieger, M., & Scholten, E. (2019). Clustering of oil droplets in o/w emulsions: Controlling cluster size and interaction strength. *Food Research International*, 122, 537–547.

**Fuhrmann, P. L**. , Kalisvaart, L. C. M. C. M., Sala, G., Scholten, E., & Stieger, M. (2019). Clustering of oil droplets in o/w emulsions enhances perception of oil-related sensory attributes. *Food Hydrocolloids*, 97, 105215.

**Fuhrmann, P. L.**, Sala, G., Stieger, M., & Scholten, E. (2020). Effect of oil droplet inhomogeneity at different length scales on mechanical and sensory properties of emulsion-filled gels: Length scale matters. *Food Hydrocolloids*, 101, 105462.

**Fuhrmann, P. L.**, Aguayo-Mendoza, M., Jansen, B., Stieger, M., & Scholten, E. (2019). Characterisation of friction behaviour of intact soft solid foods and food boli. *Food Hydrocolloids*, 100, 105441.

**Fuhrmann, P. L.**, Sala, G., Stieger, M., & Scholten, E. (2020). Influence of clustering of protein-stabilised oil droplets with proanthocyanidins on mechanical, tribological and sensory properties of o/w emulsions and emulsion-filled gels. Food Hydrocolloids, 105856.

## Manuscripts in preparation:

**Fuhrmann, P. L.**, Antuma, L., Sala, G., Stieger, M., & Scholten, E. The effect of fat-droplet clustering on the rheology and sensory perception of processed emulsion-filled particle gels

**Fuhrmann, P. L.**, Breunig, S., Sala, G., Stieger, M., & Scholten, E. Rheological behaviour of attractive emulsion gels with tuneable droplet-droplet interactions

# Overview of completed training activities

## Discipline specific courses

6th Sensory Perception & Food Preference: Affective drivers of food choice, 2016, WGS, Wageningen, the Netherlands

16th European school on Rheology, 2017, KU Leuven, Leuven, Belgium

Microscopy and Spectroscopy in Food and Plant Sciences, 2017, EPS-VLAG, Wageningen, the Netherlands

## Conferences and Symposia

16th Food Colloids Conference, 2016, Wageningen, the Netherlands

Annual European Congress on Rheology, 2018, Sorrento, Italy ([1])

Food Structure and Functionality Forum Symposium 2018, Montréal, Canada ([1,2])

17th Food Colloids Conference, 2018, Leeds, United Kingdom ([2])

5th International Conference on Food Oral Processing, 2018, Nottingham, United Kingdom ([1])

Rheology, FPH and PCC (WUR), 2018, Wageningen, the Netherlands ([1])

13th PhD Workshop on Food Engineering and Technology, 2019, Vienna, Austria ([1])

7th European Conference on Tribology, 2019, Vienna, Austria ([1])

8th International Symposium on Food Rheology and Structure, 2019, Zürich, Switzerland ([1])

Wageningen Food Symposium, AFSG (WUR), 2020, Wageningen, the Netherlands ([1])

[1]oral presentation, [2]poster presentation

## General courses

VLAG PhD week, 2016, Baarlo, the Netherlands

PhD Workshop Carousel, 2016, WGS, the Netherlands

Multivariate analysis for food data, 2016, VLAG, the Netherlands

Scientific writing, 2017, WGS, the Netherlands

Philosophy and Ethics of Food Science, 2017, WGS, the Netherlands

Career orientation, 2019, WUR, the Netherlands

## Optional courses and activities

Preparation of research proposal, 2016, Wageningen, the Netherlands

IP workshop, 2016, TiFN, Wageningen, the Netherlands

Chinese Law on Food and Agriculture (LAW-56306), 2017, Wageningen, the Netherlands

Organisation and participation PhD (FPH) study trip Singapore and Indonesia, 2018

Seminar African Philosophy and Knowledge Diversity in International Development, 2019, WASS CPT, Wageningen, the Netherlands

Project meetings TIFN "Smooth Bite for All", Wageningen, the Netherlands

Project partner visits (Unilever, FrieslandCampina, Le Groupe Bel)

Weekly group meetings Physical and Physical Chemistry of Foods, Wageningen, the Netherlands

# Colophon