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

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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 more grainy 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 more creamy (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.

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 (Akhtar, Stenzel, Murray, & Dickinson, 2005; Cutler, Morris, & Taylor, 1983; Scholten, 2017; van Aken, Vingerhoeds, & de Wijk, 2011). For emulsion-filled gels, fracture stress has been related to perceived firmness/hardness (van Vliet, van Aken, de Jongh, & Hamer, 2009; Young, Cheong, Hedderley, Morgenstern, & James, 2013; Çakır et al., 2012). 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 (Foegeding, 2007; Mao & McClements, 2012; Mosca, Rocha, Sala, van de Velde, & Stieger, 2012; Sala, van Vliet, Cohen Stuart, Aken, & van de Velde, 2009). 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, Yeomans, Norton, & Norton, 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

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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, 2015; 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, 2019; Mao & McClements, 2011) and emulsion-filled gels (Oliver et al., 2015). 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, Kalisvaart, Sala, Scholten, & Stieger, 2019). Previously, the oil droplets were clustered by mixing o/w emulsions stabilised with oppositely-charged emulsifiers (Fuhrmann et al., 2019). These electrostatic attractions in emulsions are relatively weak physical interactions, resulting in formation of oil droplet 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., 2019). 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, 2020). Using more dense 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., 2019). 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. gluaraldehyde). 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.

2. Materials and methods

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.

2.2. Sample preparation

2.2.1. 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 with single oil droplets), 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.

2.2.2. 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, single 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, single 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 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.

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/ 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 oil droplets	GSE0g	0	6 ± 1^{d}
Small clusters	GSE25g	0.25	18 ± 4^{c}
Medium clusters	GSE50g	0.50	67 ± 7^{b}
Non-clustered oild roplets, modulus matched (to GSE50g)*	GSE0_gh	0	6 ± 1^{d}

2.3. Physical characterisation of o/w emulsions and emulsion filled gels

2.3.1. 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., 2019). 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.

2.3.2. 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 mad expressly to be shown in the paper. 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 1201 × 1201 μm. Image J/Fiji (1.51s) was used to adapt the contrast and brightness of the microscopic images obtained.

2.3.3. 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 to 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):

$$\eta = K^{*}\dot{\gamma}^{n-1} \quad (1)$$

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 ($0 < n < 1$).

2.3.4. 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 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 et al. (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 (μ_{bound}), friction coefficients at 10 mm/s ($\mu_{10\ mm/s}$), 80 mm/s ($\mu_{80\ mm/s}$) and 200 mm/s ($\mu_{200\ mm/s}$) were extracted from the data. A power-law model,

$$\mu \sim speed^{b} \quad (2)$$

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

2.3.5. 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, & van Aken, 2009).

2.3.6. 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 × 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 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 was 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 vol 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.

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 et al. (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 h before collection. Saliva was centrifuged at 10,000 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 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.

2.5. Sensory evaluation

2.5.1. 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, 2016). The sensory evaluation was conducted with n = 74 untrained participants (40 female, 34 male; 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 min in randomised order. 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).

2.5.2. Sensory evaluation of emulsion-filled gels

Four emulsion-filled gels (GSE0g, GSE25g, GSE50g, GSE0_gh) were evaluated by untrained participants (n = 50, 38 female, 12 male; 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

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 et al., 2017; van Aken et al., 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.

confirm the absence of any intolerance towards the ingredients present in the emulsion-filled gels and received financial compensation upon completion of the study. Similar to 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 Eye-Question (Version 4.11.3).

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, Jaeger, & Ares, 2016) and Oppermann (Oppermann, de Graaf, Scholten, Stieger, & Piqueras-Fiszman, 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, a 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.

3. Results and discussion

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 3.2. With increasing concentration of proanthocyanidins from 0 to 0.75g proanthocyanidins per g protein, average cluster size ($D_{4,3}$) of o/w emulsions increased from 3 μm for single droplet o/w emulsions to 110 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., 2019). In addition, it has already been shown that these clusters are more dense than weakly aggregated clusters (Fuhrmann et al., 2019). 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 single droplet 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 single droplet emulsions (0.09). This increase in μ_{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., 2019). However, as such clusters aggregated by GSE are more strong and also larger than weak clusters, the friction coefficient of the emulsions can also increase again when the clusters becomes 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

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	[D4,3]	Consistency K	Flow index n	μ_{bound}	$\mu_{10\ mm/s}$	$\mu_{80\ mm/s}$	$\mu_{200\ mm/s}$	b
		(μm)	mPa s^n	(–)	(–)	(–)	(–)	(–)	(/mm/s)
O/w emulsions without saliva									
Non-clustered, single 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, single 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

o/w emulsions, as expected, changed both flow properties and lubrication behaviour and that these properties depend on cluster size.

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 et al., 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. Single oil droplets and small clusters have a large surface area, which facilitates this interaction. Therefore, single 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 as a result 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.

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. Emulsions with clusters were perceived significantly thicker than single droplet emulsions (GSE0) with the same oil volume fraction. The increase in thickness is in line with the increase in viscosity of o/w

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 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 single droplet 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 single droplet 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 et al., 2019). This suggests that the disintegration of the clusters or their deformability are important factors with regards to possitive sensory evaluation. The deformability of these clusters may be lower than that of weak clusters, since the dense structure, as a result 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 (Fig. 1). 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 Fig. 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 single droplet 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. As a consequence, 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 a partial least squares regression (PLSR) to investigate further whether the physical properties correlate with sensory properties of o/w emulsions with clustered oil droplets (Fig. 2). As expected, cluster size (D4,3), viscosity (K) and perceived thickness are highly correlated. It can be seen that 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

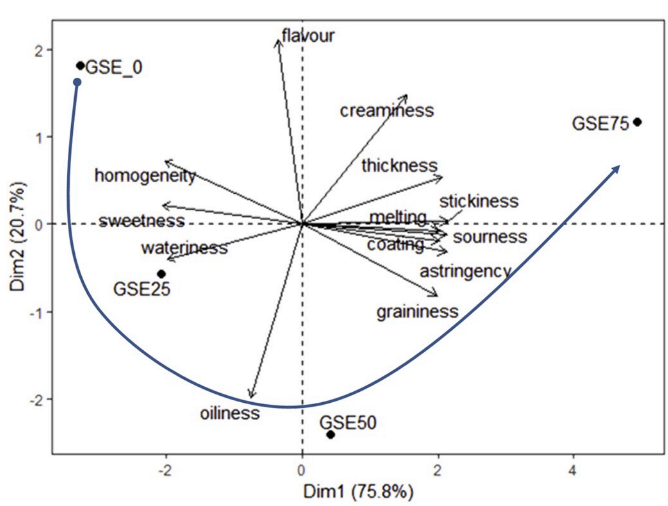

Fig. 1. PCA biplot of o/w emulsions with clustered oil droplets and sensory attributes determined using RATA ($n_{participants} = 74$). The blue arrow is added to guide the reader on how cluster size increases throughout the PCA biplot. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

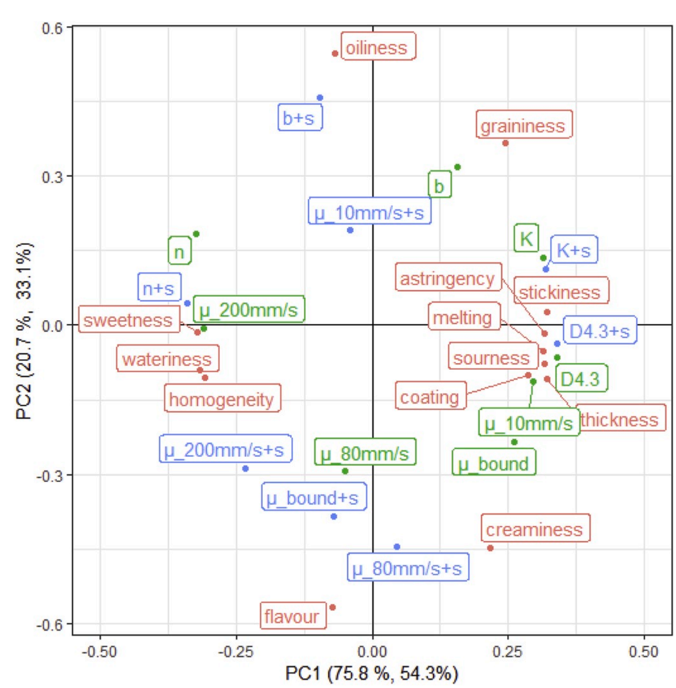

Fig. 2. PLSR plot of sensory attributes of o/w emulsions (with and without clustered oil droplets) (in red), emulsion properties (green) and emulsion properties after the addition of saliva ("+s"; blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

two entrained surfaces. We observe that o/w emulsions that were rated as more oily 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., 2019), 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.

3.4. Mechanical properties of emulsion-filled gels containing oil droplet clusters

Emulsion-filled gels with single oil droplets (GSE0g, Fig. 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 examples, a micrograph of a gel with medium-sized oil droplet clusters (GSE50g) is shown in Fig. 3B. As can be seen, the clusters remained stable in the gel. In agreement with our earlier characterization (Fuhrmann et al., 2019), the clusters were dense and randomly-shaped.

Incorporating the strongly-clustered o/w emulsions into a gelatine gel matrix changed Young's modulus, fracture stress and fracture strain (Table 5). Without oil droplets, Young's modulus of the unfilled gel was 10 kPa. Introducing non-clustered, single 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 is in agreement with previous studies (Oliver et al., 2015). 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 et al., 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.

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 Fig. 4. 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.

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 Fig. 5. To relate these sensory attributes to the physical properties of the emulsion-filled gels, we performed a partial least squares regression (PLSR), shown in Fig. 6.

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) (Fig. 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

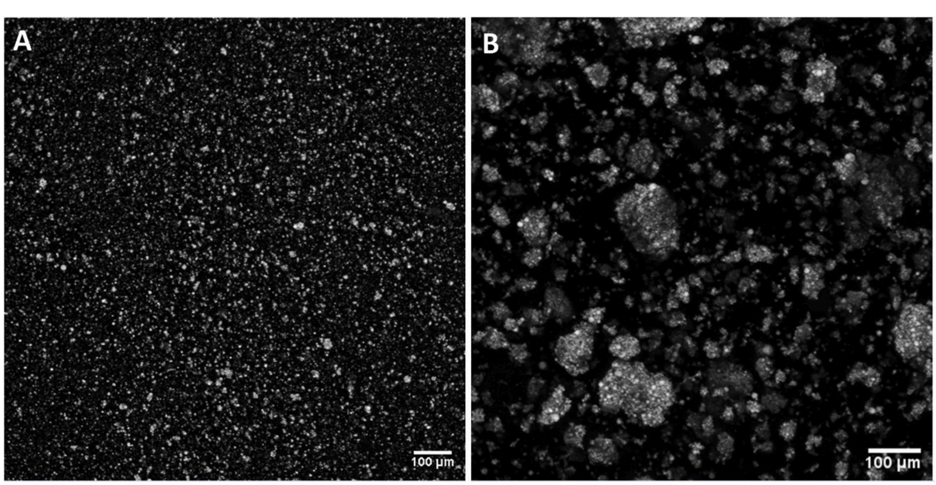

Fig. 3. CLSM micrographs of emulsion-filled gels with an oil volume fraction of 0.01. (A) non clustered oil droplets (GSE0g) (red), (B) medium-sized clusters (GSE50g) (only oil phase is shown). Scale bar represents 100 μm.

Table 5
Young's modulus, true fracture strain and stress of emulsion-filled gels with single oil droplets, with single droplets and increased modulus, and with oil droplet clusters (small clusters, medium clusters). *relative to 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, single 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, single oil droplets	GSE0_gh	6±1[c]	16.1 ± 0.9[ab]	–	1.2 ± <0.1[a]	31.4 ± 1.1[a]

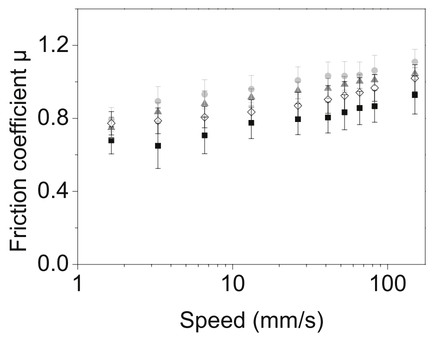

Fig. 4. Friction coefficient between emulsion filled gels (GSE0g, GSE25g, GSE50g and GSE0_gh) and a PDMS probe as a function of sliding speed (black squares: GSE0g, open diamond: GSE0_gh, grey triangle: GSE50g and grey circle: GSE25g). Measurements were performed in triplicates. Error bars represent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

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) (Fig. 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, Jiao, & Guo, 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, Piqueras-Fiszman, de Graaf, Scholten, & Stieger, 2016).

The gels with clustered oil droplets were, furthermore, ranked as

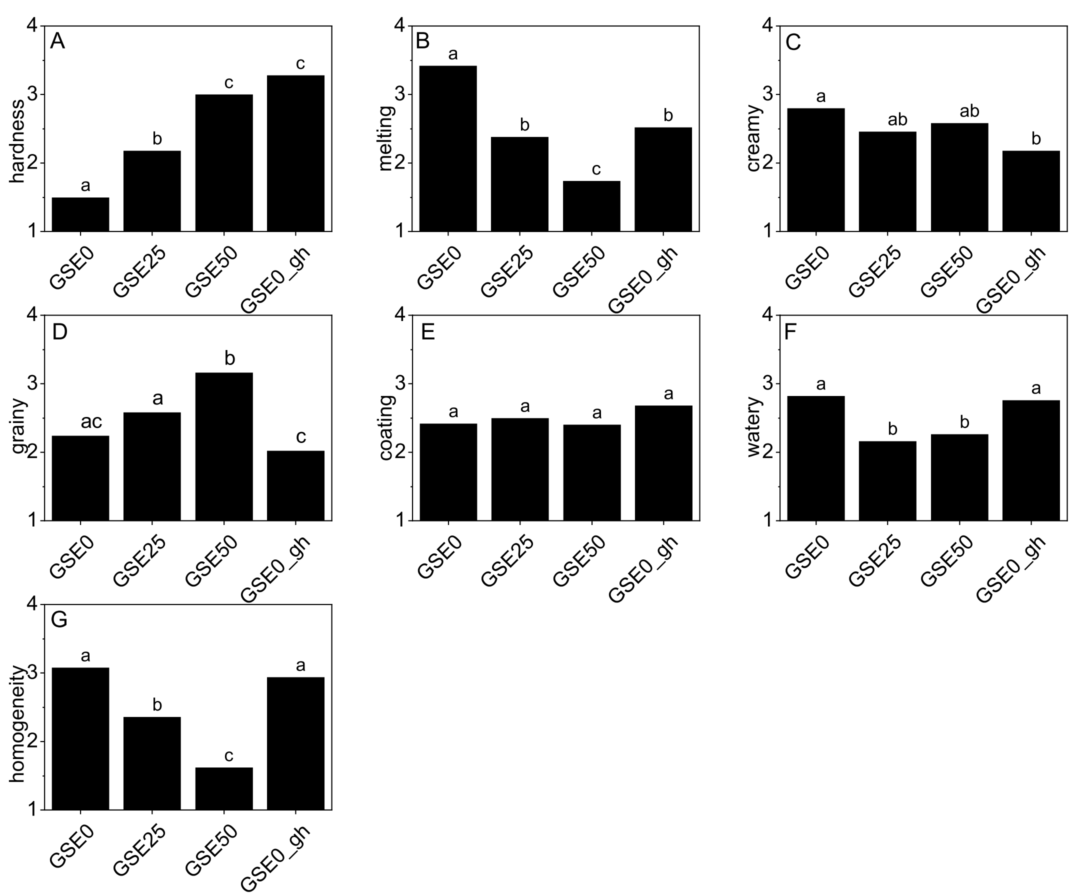

Fig. 5. Average ranks ($n_{participants} = 50$) of emulsion-filled gels with single and with clustered oil droplets (GSE0g, GSE25g, GSE50g and GSE0_gh) for the attributes A) hardness B) melting C) creamy D) grainy E) coating F) watery and G) homogeneity. Different average ranks of emulsion-filled gels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

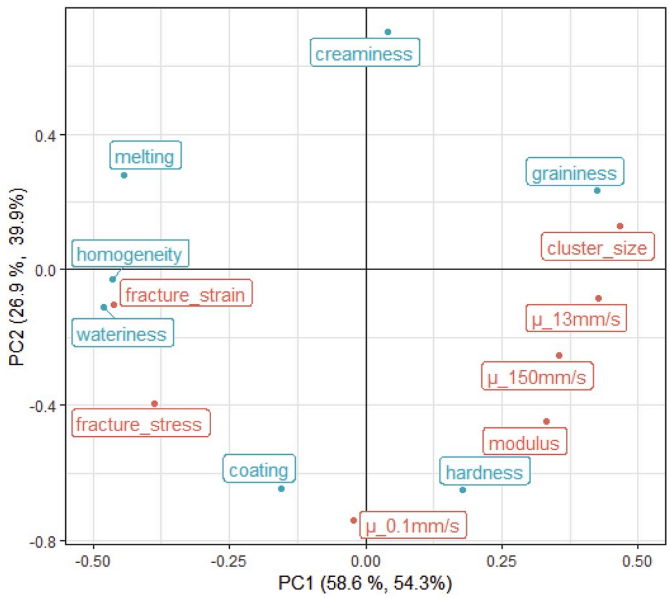

Fig. 6. PLSR plot of sensory attributes of emulsion filled gels (with and without GSE) (in red), gel properties (blue). Principal components 1 and 2 of the sensory attributes explain 85% of the variance, while PC 1 and 2 of the gel properties explain 94% of the variance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

grainier than gels with non-clustered droplets (Fig. 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 (Fig. 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 incorporation of weaker clusters into emulsion-filled gels have shown that less dense clusters are not detected (Fuhrmann et al., 2020). This relation is confirmed in the PLSR (Fig. 6), where cluster size and graininess are located near each other. Secondly, we observe a correlation (Fig. 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 unclustered 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 more grainy due to the presence of a large number of stiff gel pieces. The results of the attribute homogeneity are in line with the changes in graininess.

Creaminess (Fig. 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 (Fig. 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 (Fig. 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. It is clear that 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 an useful approach to change the microstructure of emulsion-filled gels and manipulate their mechanical and sensorial properties.

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. As a result 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 perception of thickness, wateriness, creaminess and coating. Cluster strength should not be too high to prevent graininess perception.

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Declaration of competing interest

The authors have declared that no competing interests exist.

CRediT authorship contribution statement

Philipp L. Fuhrmann: Investigation, Conceptualization, Writing - original draft. **Guido Sala:** Funding acquisition, Supervision, Writing - review & editing. **Elke Scholten:** Funding acquisition, Supervision, Writing - review & editing. **Markus Stieger:** Project administration, Funding acquisition, Supervision, Writing - review & editing.

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References

van Aken, G. A., Oliver, L., & Scholten, E. (2015). Rheological effect of particle clustering in gelled dispersions. *Food Hydrocolloids, 48*, 102–109.
van Aken, G. A., Vingerhoeds, M. H., & de Wijk, R. A. (2011). Textural perception of liquid emulsions: Role of oil content, oil viscosity and emulsion viscosity. *Food Hydrocolloids, 25*(4), 789–796.
Akhtar, M., Stenzel, J., Murray, B. S., & Dickinson, E. (2005). Factors affecting the perception of creaminess of oil-in-water emulsions. *Food Hydrocolloids, 19*(3), 521–526.
Benjamins, J., Vingerhoeds, M. H., Zoet, F. D., de Hoog, E. H. A., & van Aken, G. A. (2009). Partial coalescence as a tool to control sensory perception of emulsions. *Food Hydrocolloids, 23*(1), 102–115.
Bohin, M. C., Vincken, J. P., Van Der Hijden, H. T. W. M., & Gruppen, H. (2012). Efficacy of food proteins as carriers for flavonoids. *Journal of Agricultural and Food Chemistry, 60*(16), 4136–4143.
Bohin, M. C., Vincken, J. P., Westphal, A. H., Tripp, A. M., Dekker, P., van der Hijden, H. T., et al. (2014). Interaction of flavan-3-ol derivatives and different caseins is determined by more than proline content and number of proline repeats. *Food Chemistry, 158*, 408–416.
Bongaerts, J. H. H., Rossetti, D., & Stokes, J. R. (2007). The lubricating properties of human whole saliva. *Tribology Letters, 27*(3), 277–287.
Çakır, E., Daubert, C. R., Drake, M. A., Vinyard, C. J., Essick, G., & Foegeding, E. A. (2012). The effect of microstructure on the sensory perception and textural characteristics of whey protein/κ-carrageenan mixed gels. *Food Hydrocolloids, 26*(1), 33–43.
Charlton, A., Baxter, N., Khan, M., Moir, A., Haslam, E., Davies, A., & Williamson, M. (2002). Polyphenol/peptide binding and precipitation. *Journal of Agricultural and Food Chemistry, 50*(6), 1593–1601.
Cutler, A. N., Morris, E. R., & Taylor, L. J. (1983). Oral perception of viscosity in fluid foods and model systems. *Journal of Texture Studies, 14*(4), 377–395.
Dresselhuis, D. M., de Hoog, E. H. A., Cohen Stuart, M. A., & van Aken, G. A. (2008). Application of oral tissue in tribological measurements in an emulsion perception context. *Food Hydrocolloids, 22*(2), 323–335.
Foegeding, E. A. (2007). Rheology and sensory texture of biopolymer gels. *Current Opinion in Colloid & Interface Science, 12*(4–5), 242–250.
Fuhrmann, P. L., Kalisvaart, L. C. M., Sala, G., Scholten, E., & Stieger, M. (2019b). Clustering of oil droplets in o/w emulsions enhances perception of oil-related sensory attributes. *Food Hydrocolloids*, 105215.
Fuhrmann, Philipp L., Sala, G., Stieger, M., & Scholten, E. (2019a). Clustering of oil droplets in o/w emulsions: Controlling cluster size and interaction strength. *Food Research International, 122*, 537–547.
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.
Gong, J. P. (2006). Friction and lubrication of hydrogels—its richness and complexity. *Soft Matter, 2*(7), 544–552.
González-Martín, M. I., Severiano-Pérez, P., Revilla, I., Vivar-Quintana, A. M., Hernández-Hierro, J. M., González-Pérez, C., et al. (2011). Prediction of sensory attributes of cheese by near-infrared spectroscopy. *Food Chemistry, 127*(1), 256–263.
Imai, E., Hatae, K., & Shimada, A. (1995). Oral perception of grittiness: Effect of particle size and concentration of the dispersed particles and the dispersion medium. *Journal of Texture Studies, 26*(5), 561–576.
Khouryieh, H., Puli, G., Williams, K., & Aramouni, F. (2015). Effects of xanthan–locust bean gum mixtures on the physicochemical properties and oxidative stability of whey protein stabilised oil-in-water emulsions. *Food Chemistry, 167*, 340–348.
Laguna, L., Farrell, G., Bryant, M., Morina, A., & Sarkar, A. (2017). Relating rheology and tribology of commercial dairy colloids to sensory perception. *Food & Function, 8*(2), 563–573.
Lett, A. M., Yeomans, M. R., Norton, I. T., & Norton, J. E. (2016). Enhancing expected food intake behaviour, hedonics and sensory characteristics of oil-in-water emulsion systems through microstructural properties, oil droplet size and flavour. *Food Quality and Preference, 47*, 148–155.
Liu, H., Jiao, Z., & Guo, S. (2014). Effect of epigallocatechin gallate on the properties of gelatin. *International Journal of Food Properties, 17*(10), 2119–2130.
Liu, K., Stieger, M., van der Linden, E., & van de Velde, F. (2015). Fat droplet characteristics affect rheological, tribological and sensory properties of food gels. *Food Hydrocolloids, 44*, 244–259.
Maier, C., Oechsle, A. M., & Weiss, J. (2015). Cross-linking oppositely charged oil-in-water emulsions to enhance heteroaggregate stability. *Colloids and Surfaces B: Biointerfaces, 135*, 525–532.
Mao, Y., & McClements, D. J. (2011). Modulation of bulk physicochemical properties of emulsions by hetero-aggregation of oppositely charged protein-coated lipid droplets. *Food Hydrocolloids, 25*(5), 1201–1209.
Mao, Y., & McClements, D. J. (2012). Modulation of emulsion rheology through electrostatic heteroaggregation of oppositely charged lipid droplets: Influence of particle size and emulsifier content. *Journal of Colloid and Interface Science, 380*(1), 60–66.
Matalanis, A., & McClements, D. J. (2012). Factors influencing the formation and stability of filled hydrogel particles fabricated by protein/polysaccharide phase separation and enzymatic cross-linking. *Food Biophysics, 7*(1), 72–83.
Meyners, M., Jaeger, S. R., & Ares, G. (2016). On the analysis of Rate-All-That-Apply (RATA) data. *Food Quality and Preference, 49*, 1–10.
Mosca, A. C., Rocha, J. A., Sala, G., van de Velde, F., & Stieger, M. (2012). Inhomogeneous distribution of fat enhances the perception of fat-related sensory attributes in gelled foods. *Food Hydrocolloids, 27*(2), 448–455.
Oliver, L., Berndsen, L., van Aken, G. A., & Scholten, E. (2015). Influence of droplet clustering on the rheological properties of emulsion-filled gels. *Food Hydrocolloids, 50*, 74–83.
Oliver, L., Wieck, L., & Scholten, E. (2016). Influence of matrix inhomogeneity on the rheological properties of emulsion-filled gels. *Food Hydrocolloids, 52*, 116–125.
Oppermann, A. K. L., de Graaf, C., Scholten, E., Stieger, M., & Piqueras-Fiszman, B. (2017). Comparison of Rate-All-That-Apply (RATA) and Descriptive sensory Analysis (DA) of model double emulsions with subtle perceptual differences. *Food Quality and Preference, 56*, 55–68. Part A.
Oppermann, A. K. L., Piqueras-Fiszman, B., de Graaf, C., Scholten, E., & Stieger, M. (2016). Descriptive sensory profiling of double emulsions with gelled and non-gelled inner water phase. *Food Research International, 85*, 215–223.
Ostwald, W. (1925). Ueber die Geschwindigkeitsfunktion der Viskosität disperser Systeme. II. *Kolloid Zeitschrift, 36*(3), 157–167.
Ozdal, T., Capanoglu, E., & Altay, F. (2013). A review on protein–phenolic interactions and associated changes. *Food Research International, 51*(2), 954–970.
Pascal, C., Poncet-Legrand, C., Imberty, A., Gautier, C., Sarni-Manchado, P., Cheynier, V., et al. (2007). Interactions between a non glycosylated human proline-rich protein and flavan-3-ols are affected by protein concentration and polyphenol/protein ratio. *Journal of Agricultural and Food Chemistry, 55*(12), 4895–4901.
Sala, G., de Wijk, R. A., van de Velde, F., & van Aken, G. A. (2008). Matrix properties affect the sensory perception of emulsion-filled gels. *Food Hydrocolloids, 22*(3), 353–363.
Sala, G., Van Aken, G. A., Stuart, M. A. C., & Van De Velde, F. (2007). Effect of droplet-matrix interactions on large deformation properties of emulsion-filled gels. *Journal of Texture Studies, 38*(4), 511–535.
Sala, G., van Vliet, T., Cohen Stuart, M. A., Aken, G. A. van, & van de Velde, F. (2009). Deformation and fracture of emulsion-filled gels: Effect of oil content and deformation speed. *Food Hydrocolloids, 23*(5), 1381–1393.
Sarkar, A., Andablo-Reyes, E., Bryant, M., Dowson, D., & Neville, A. (2019). Lubrication of soft oral surfaces. *Current Opinion in Colloid & Interface Science, 39*, 61–75. February 1).
Sarkar, A., Goh, K. K. T., & Singh, H. (2009). Colloidal stability and interactions of milk-protein-stabilized emulsions in an artificial saliva. *Food Hydrocolloids, 23*(5), 1270–1278.
Scholten, E. (2017). Composite foods: From structure to sensory perception. *Food & Function, 8*(2), 481–497.
Selway, N., & Stokes, J. R. (2013). Insights into the dynamics of oral lubrication and mouthfeel using soft tribology: Differentiating semi-fluid foods with similar rheology. *Food Research International, 54*(1), 423–431.
Silletti, E., Vingerhoeds, M. H., Norde, W., & van Aken, G. A. (2007). The role of electrostatics in saliva-induced emulsion flocculation. *Food Hydrocolloids, 21*(4), 596–606.
van Vliet, T., van Aken, G. A., de Jongh, H. H. J., & Hamer, R. J. (2009). Colloidal aspects of texture perception. *Advances in Colloid and Interface Science, 150*(1), 27–40.
Wium, H., Qvist, K. B., & Gross, M. (1997). Uniaxial compression of UF-Feta cheese related to sensory texture analysis. *Journal of Texture Studies, 28*(4), 455–476.
Young, A. K., Cheong, J. N., Hedderley, D. I., Morgenstern, M. P., & James, B. J. (2013). Understanding the link between bolus properties and perceived texture. *Journal of Texture Studies, 44*(5), 376–386.