

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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ARTICLE INFO

Keywords:

Oil droplet clustering

Oil distribution

Emulsion-filled gels

Fat-related perception

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 an 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, whereas 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. 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, whereas 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.

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., 2007). 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 (Chojnicka, Sala, de Kruif, & van de Velde, 2009; Lett, Norton, & Yeomans, 2016; Sala, van Aken, Stuart, & van de Velde, 2007).

Additionally, the spatial distribution of oil droplets within an

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<https://doi.org/10.1016/j.foodhyd.2019.105462>

Received 28 August 2019; Received in revised form 18 October 2019; Accepted 23 October 2019

Available online 25 October 2019

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emulsion-filled gel influences the mechanical properties. Oliver, Wieck, and Scholten (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 et al., 2016; Oliver, Berndsen, van Aken, & Scholten, 2015). 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, Rocha, Sala, van de Velde, and Stieger (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-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.

2. Materials and methods

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 (TiO_2) was purchased from Minerals Water (Rainham, United Kingdom).

2.2. Oil-in-water emulsions

2.2.1. Single droplet o/w emulsions

2.2.1.1. 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 h 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-homogenising with a rotor-stator homogeniser (Ultra-Turrax T25, IKA, Germany) at 8000 rpm for 3 min. 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.

2.2.1.2. 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 min 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 min. The emulsion was further prepared as explained in the previous section.

2.2.1.3. 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 min. 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.

2.2.2. 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 single droplet emulsions, samples were stirred, using a magnetic stirrer, and subsequently stored for 24 h 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 single droplet 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.

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 min. 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 h before further processing. Before measurements, gels were equilibrated at room temperature for 2 h. 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.

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 2 h. Subsequently, the suspension was heated to 80 °C for 30 min 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 $1 \times 1 \times 5$ 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, at 30 °C. 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 phase, abbreviated as 17.5/17.5. An overview and a schematic drawing of the prepared systems can be found in Table 2.

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) TiO₂ was added to the aqueous phase of the gels that contained 5% (v/v) oil, and 0.005% (w/w) TiO₂ 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.

2.5. Structural, physical and mechanical properties of emulsions and emulsion-filled gels

2.5.1. Light microscopy and CLSM

Light microscopy pictures (Fig. 1A–C) and CLSM pictures (Fig. 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, Mannheim, 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 512 \times 512 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.

2.5.2. 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

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. Oil volume fraction in the gels: 20% (v/v). 150 mM NaCl in all gels.

Sample	Sample code	Fraction gelatine-stabilised emulsion	Fraction DATEM-stabilised emulsion	Average droplet/cluster size [μm , in gel]
Homogeneous distribution	GD10	1	0	2.5
Homogeneous distribution	GD01	0	1	2
Small clusters	GD28	0.2	0.8	10
Large clusters	GD55	0.5	0.5	20

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	1:1	
Heterogeneous (with equal oil conc.)	17.5/17.5	17.5	17.5	17.5		
Heterogeneous	5/30	17.5	5	30		

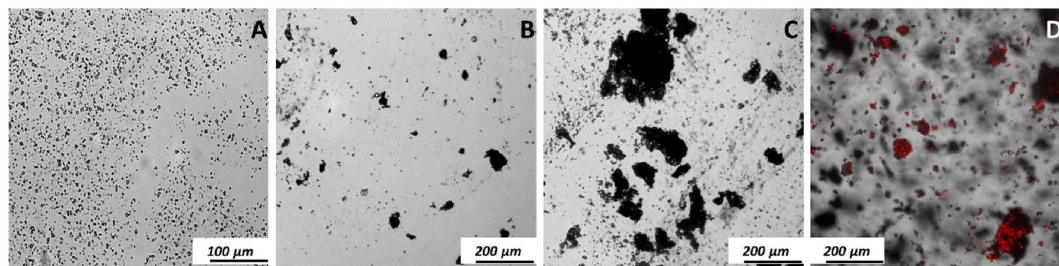


Fig. 1. Micrographs of low acyl gellan gels with A) gelatine-stabilised emulsion, B) GD28 emulsion with small clusters, C) and D) clustered emulsion with large clusters (GD55). Samples A-C are light microscopy images, sample D is a CLSM image. In sample D, the oil phase was stained with Nile Red. Scale 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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.

2.5.3. 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.

2.5.4. 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, Oliver, and Scholten (2015) for gels with an inhomogeneous droplet distribution. The relative modulus E_r (modulus of the mixed gel relative to the modulus of the matrix) is related to the Poisson ratio of the oil droplets, ν_m , the volume fraction, ϕ , 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, χ as

$$E_r = \frac{15(1 - \nu_m)(M - 1)\chi\phi_{total}}{(8 - 10\nu_m)M + 7 - 5\nu_m - (8 - 10\nu_m)(M - 1)\chi\phi_{total}} + 1 \quad (1)$$

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

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 ($n_{\text{gel-in-gel}} = 50$, $n_{\text{hetero-aggregates}} = 56$, 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 min each. During each session, participants evaluated 3 or 4 samples with breaks of 2 min 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 to 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, de Graaf, Scholten, Stieger, and Piqueras-Fiszman (2017); van Aken, Vingerhoeds, and de Wijk (2011) and Benjamins, Vingerhoeds, Zoet, de Hoog, and van Aken (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.

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 significance level.

3. Results and discussion

3.1. Effect of oil droplet clustering at a μm -scale

3.1.1. 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 single droplet 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 (Fig. 1). 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 Fig. 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 $20.5 \pm 5.2 \mu\text{m}$, which was smaller than the $50 \mu\text{m}$ 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

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. A chocolate cookie with pieces or nuts is for example not homogeneous.
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

average size of $10.4 \pm 1.7 \mu\text{m}$, which was comparable to the size of the clusters when present in the o/w emulsions ($8 \mu\text{m}$). 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.

3.1.2. 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 single droplet 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, van Aken, Stuart, & Van De Velde, 2007; van Vliet, 1988). In the case of gels with DATEM-stabilised 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).

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 (1995), 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, Scholten, and van Aken (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-stabilised 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, Prosapio, Norton, & Mills, 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

Table 4

Mechanical properties of gels with incorporated (hetero-aggregated) oil droplet clusters. *This value was obtained from literature (Cassanelli et al., 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

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 et al., 2007). 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.

3.1.3. Sensory properties

As mentioned in section 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 (Fig. 2). An overview of the gels tested can be found in Tables 1 and 4.

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, Stieger, van der Linden, & van de Velde, 2015), (ii) the fracture properties of the mixed gel (Sala, de Wijk, van de Velde, & van Aken, 2008) and (iii) the melting behaviour of the matrix (Sarkar, Ye, & Singh, 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,

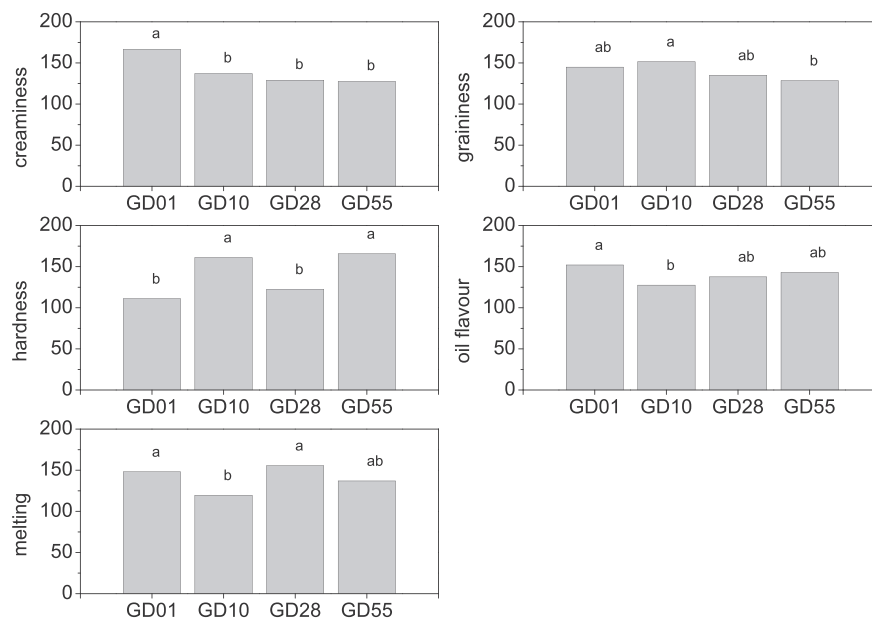


Fig. 2. Rank sum of attributes of emulsion-filled gels with DATEM-stabilised emulsions (GD01), gelatine-stabilised emulsions (GD10), emulsions with small clusters (GD28) and large clusters (GD55). Only attributes with significant differences ($p < 0.05$) among samples are shown. The RATA intensity data was transformed into rank orders and analysed using a non-parametric Friedman test.

Van den Broek, Van de Velde, & Stieger, 2015). 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 was used in the other studies). In addition, also the melting properties may have an influence on 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 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 mechanical properties of the gels are dominated by the interactions between emulsifier and matrix. 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.

3.2. Effect of oil droplet inhomogeneity at a mm-scale

3.2.1. 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. 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. (2007). 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 σ_r 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, σ_r , is related to the volume fraction, ϕ , of the fillers as

$$\sigma_r = \frac{\left(1 - \frac{\phi}{\phi_g}\right)^{\frac{1}{3}}}{\left(1 - \frac{\phi}{\phi_g}\right)^{\frac{2}{3}}} \quad (2)$$

where the oil droplets are the fillers, and ϕ_g is the maximum packing fraction, 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.

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}

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 et al., 2007).

3.2.1.1. 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., 2007).

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 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 a significant change in the brittleness, so a change in deformability or elasticity. This result is in line with literature (Brownsey, Ellis, Ridout, & Ring, 1987; Sala et al., 2007).

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.

3.2.2. Sensory properties

As mentioned in section 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 Fig. 3. Hardness, oiliness, oil flavour, homogeneity, lingering, creaminess, graininess, stickiness and mouth-coating differed ($p < 0.05$) among the gel-in-gels.

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, whereas 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 et al., 2007). Unexpectedly, gel-in-gels containing the lowest oil concentration in the

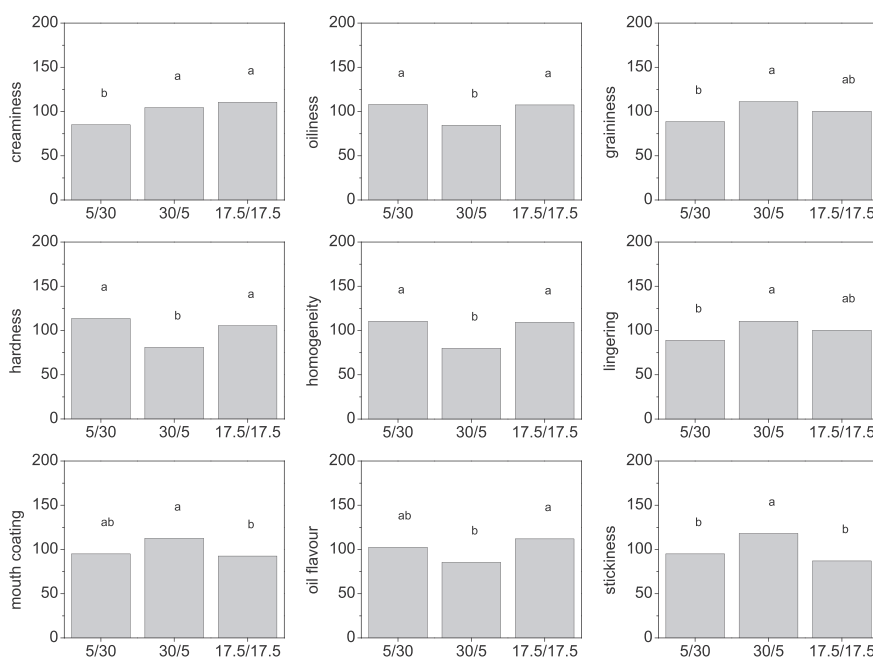


Fig. 3. Rank sum of attributes of gel-in-gels. Gel-in-gels with 5% (v/v) oil in the particles and 30% (v/v) oil in the continuous phase are denoted as 5/30, gel-in-gels with 30% (v/v) oil in the particles and 5% (v/v) oil in the continuous phase as 30/5, and gel-in-gels with 17.5% (v/v) oil in both the 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.

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, Molnes, Haug, and Draget (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, which may 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 is in contrast to 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 of the oil droplet distribution can change texture-related attributes such as hardness, but also fat-related sensations, such as oiliness and coating perception.

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 has to be careful with generalisations, as it has to 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. Yet, 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. 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 a 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.

Financial disclosure

The project is organised by and executed under the auspices of TiFN, a public-private partnership on precompetitive research in food and nutrition. Funding for this research was obtained from FrieslandCampina, Fromageries Bel, and Unilever, the Netherlands Organisation for Scientific Research and the Top-sector Agri & Food.

Declaration of competing interest

The authors have declared that no competing interests exist.

Acknowledgements

We want to thank Gijs Appels and Qinyuan Li for their help with the execution of experimental work.

Appendix A. Supplementary material

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 et al., 2015).

The modulus of the total gel, E_{gel} , can then be expressed as:

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

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, ϕ 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 ϕ_{inner} is the volume fraction of fillers within the clusters, and ϕ_{outer} the volume fraction of non-clustered fillers in the continuous phase. χ is the ratio between fillers in the outer matrix, ϕ_{outer} and the total filler volume fraction, ϕ_{total} . The volume fraction of the clusters can then be described by:

$$\phi_{cluster} = \frac{\phi_{total}(\chi - 1)}{\phi_{total} - \phi_{inner}} \text{ and } \chi = \frac{\phi_{outer}}{\phi_{total}} \quad (A2)$$

These clusters have a modulus defined as:

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

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

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

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

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 taking into account the interfacial tension, γ_{ow} , and the size of the oil droplets as $E_{filler} = \frac{6\gamma_{ow}}{r}$. Taking a surface tension (γ_{ow}) for gelatine-stabilised 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-stabilised 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 et al., 2015).

Appendix B. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2019.105462>.

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