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

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ABSTRACT

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

1. Introduction

Fat is an essential ingredient of many foods, such as milk, yogurt, and mayonnaise. Fat composition and oil droplet size largely influence the rheological properties of emulsions and therefore have a large effect on sensory perception of o/w emulsion-based foods. A critical parameter for the rheological properties of o/w emulsions is the spatial distribution of oil droplets (Mao & McClements, 2012b; Mosca, Rocha, Sala, van de Velde, & Stieger, 2012; Sala, van Vliet, Cohen Stuart, van Aken, & van de Velde, 2009). The spatial distribution of oil droplets in an o/w emulsion can be uniform (homogeneous), or non-uniform (inhomogeneous) with macro- or microscopic oil-enriched and oil-depleted areas (Oliver, Berndsen, van Aken, & Scholten, 2015; Oliver, Wieck, & Scholten, 2016). On a microscopic scale, an inhomogeneous spatial distribution of oil droplets is obtained when oil droplets cluster. Here, clustering is referred to as the purposefully obtained assembly of multiple oil droplets into clusters with specific and controllable cluster size. Clustering of oil droplets has been shown to cause significant changes in the rheological properties of liquid emulsions (Mao & McClements, 2011). There are several reasons for these changes in rheological properties resulting from droplet clustering. The entrapment of continuous aqueous phase in the oil droplet cluster causes a strong increase in viscosity in liquid systems, since the effective volume fraction of the oil phase increases to a large extent McClements (2012). Furthermore, clusters of oil droplets are typically non-spherical objects, which increases the hydrodynamic diameter. As non-spherical objects occupy larger volumes than spherical objects (Marangoni, 2000), the viscosity increases. Additionally, clustering of oil droplets can influence

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the effective stiffness of oil droplets, as droplet-droplet interactions are introduced. In clustered droplets, additional elasticity is conferred due to bonds between droplets and droplet-droplet friction (Filip et al., 2006). The clustering of oil droplets thus changes the structure and rheological properties of o/w emulsions. Modulation of oil droplet clustering can thus be used as an approach to tailor rheological properties of liquid oil-containing foods, such as emulsion-based beverages (i.e. milk drinks), salad dressings and cream liqueurs, and might consequently be used to reformulate foods. It is desired to control cluster size and interaction strength within a cluster and between clusters to obtain o/w emulsions with defined rheological properties.

To induce droplet clustering, several approaches seem promising and were investigated in this study. Oil droplets are frequently stabilised by proteins, and protein-protein interactions can be used to cluster oil droplets. When proteins are located at the o/w interface of oil droplets, clustering between oil droplets can occur, provided that the electrostatic repulsion between droplets is overcome by attractive interactions. Furthermore, the occurrence of clustering depends on the volume fraction of the oil droplets. With increasing volume fraction of oil droplets, clustering is facilitated due to a higher probability of droplet-droplet contacts and interactions. Specific compounds are able to induce or facilitate protein-protein interactions. Various compounds belonging to the group of proanthocyanidins have been shown to induce complexation with proteins, such as whey proteins or caseins, which leads to clustering of the proteins (Bohin et al., 2014; Bohin, Vincken, Van Der Hijden, & Gruppen, 2012). There is a broad range of specific interactions responsible for complexation ranging from hydrogen bonds, hydrophobic interactions to π-π stacking and covalent bonds. Currently, it is not known whether proanthocyanidins are able to induce oil droplet clustering of protein-stabilised droplets. Protein-protein interactions can also be induced by enzymatic cross-linking. Peroxidase has been reported to be able to crosslink proteins, as well as proteins with other polymers. (Liu et al., 2015, 2017; Matheis & Whitaker, 1984). Also transglutaminase is able to create bonds between proteins (Gaspar & de Góes-Favoni, 2015; Zeeb et al., 2013), so it seems plausible that clusters of protein-stabilised oil droplets can be obtained using transglutaminase.

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

2. Materials and methods

2.1. Materials

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

2.2. Preparation of clustered oil droplets in o/w emulsions

2.2.1. WPI-stabilised o/w emulsions with proanthocyanidins

An overview of the prepared o/w emulsions can be found in Table 1. O/w emulsions were prepared with an aqueous phase containing WPI (3.75, 1.88 and 0.94 g/L final WPI concentration in the aqueous phase) in a 0.12 M McIlvaine buffer at pH 3. This pH was previously identified to be the optimal pH for interactions between certain proanthocyanidins and proteins (Bohin et al., 2014; Naczk, Oickle, Pink, & Shahidi, 1996; Rawel, Meidtner, & Kroll, 2005). After complete dissolution, sunflower oil was added to WPI solutions. O/w emulsions had a final oil concentration of 5%, 10% and 20% (*v*/v). The mixtures were pre-emulsified with a rotor-stator homogenizer (Ultra-Turrax, T25, IKA, Germany) at 8000 rpm for 3 min. Subsequently, pre-emulsions were homogenised at 180 bar for 4 cycles (LabhoScope, Delta Instruments, The Netherlands). As a source of proanthocyanidins, grape seed extract Vitaflavan® was used. Clustered emulsions were prepared by combining various volumes of grape seed extract (GSE) stock solutions (200 g/L GSE) with WPI-stabilised o/w emulsions (5, 10 or 20% (v/v) oil). Between 0.25 g and 0.75 g GSE per gram emulsifying protein (GSE concentration in the final emulsion) was used to obtain emulsions varying in cluster size. We denote this as 25%, 50% and 75% GSE. Dilution effects due to the addition of GSE stock solutions were considered negligible. Following the addition of GSE stock solutions, o/w emulsions were vigorously mixed, by shaking, and stored for 24 h at room temperature to allow oil droplet clustering to occur.

2.2.2. Hetero-aggregation of WPI stabilised o/w emulsions

An overview of the prepared o/w emulsions can be found in Table 1. First, stock solutions of WPI, gelatine, and DATEM were prepared. WPI at a concentration of 6.4 g/L was added to a 7.5 mM citric acid solution at pH 7 (set with 1 M NaOH). Citric acid was used as a food-grade chelating agent, to avoid influence of divalent ions on clustering. The solution was stirred with a magnetic stirrer for at least 2 h to allow complete hydration of the protein. Gelatine solutions were prepared at a concentration of 20 g gelatine/L aqueous phase by addition of gelatine to a citric acid solution of pH 5 and 7 (set with 1 M NaOH). The gelatine dispersion was heated to 80 °C for 30 min whilst stirring to dissolve the gelatine. DATEM solutions were prepared by dissolving DATEM into sunflower oil at a concentration of 4 g/L oil. Solutions were heated to 80 °C for 10 min to dissolve DATEM. Both gelatine and DATEM solutions were cooled to room temperature before further use. The pH of the gelatine solutions was set, if required, to values of 5 or 7

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

Emulsions	Cluster	Final oil concentration [%]	Final emulsifier concentration [g/L emulsion]	Emulsifier	GSE concentration [% per g protein]		
Clustering with proanthocyanidins							
Non-clustered emulsion	Homogeneous droplet distribution	5, 10, 20	0.9 (5% oil) 1.7 (10% oil) 3 (20% oil)	WPI	0		
Clustered emulsions							
GSE25 GSE50 GSE75	Small cluster Medium sized cluster Large cluster	5, 10, 20	0.9 (5% oil) 1.7 (10% oil) 3 (20% oil)	WPI	25 50 75		
Emulsions	*Ratio emulsions*	*Final oil concentration [%]*	*Final emulsifier concentration [g/L emulsion]*		*Emulsifier*	*Volume emulsion (to total 100 mL) [mL]*	
Hetero-aggregated emulsions							
Gelatine-Datem emulsions						*Gelatine*	*DATEM*	*WPI*
non-clustered	*00:10*	5, 10, 20	0.2 (5% oil) 0.4 (10% oil) 0.8 (20% oil)		DATEM	0	100	-
	10:00		1.5 (5% oil) 3 (10% oil) 6 (20% oil)		Gelatine	100	0	-
clustered	*01:09*	5, 10, 20	.	Gelatine	DATEM	10	90	
	02:08					20	80	-
	03:07					30	70	-
	04:06					40	60	-
	05:05					50	50	-
	06:04					60	40	-
	07:03					70	30	-
	09:01					90	10	-
Gelatine-WPI emulsions								
non-clustered	*00:10*	5, 10, 20	0.5 (5% oil) 1 (10% oil) 1.9 (20% oil)		WPI	0	-	100
	10:00		1.5 (5% oil) 3 (10% oil) 6 (20% oil)		Gelatine	100	-	0
clustered	*01:09*	5, 10, 20	.	Gelatine	WPI	10	-	90
	02:08					20	-	80
	03:07					30	-	70
	04:06					40	-	60
	05:05					50	-	50
	06:04					60	-	40
	07:03					70	-	30
	09:01					90	-	10

with a 1 M NaOH solution. Aqueous and oil phases were combined to prepare single emulsions with an oil volume fraction of 0.4. The mixtures were pre-emulsified and emulsified as described in 2.2.1. Emulsions were subsequently diluted to final oil concentrations of 5, 10 and 20% (equal to an oil volume fraction of 0.05, 0.10 and 0.20). Hetero-aggregated emulsions were prepared by combining two single emulsions under stirring at room temperature at different volumetric ratios. The selected volume ratios were 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 9:1, and 10:0. Gelatine-stabilised emulsions (positively charged) were mixed with either WPI- or DATEM-stabilised (negatively charged) o/w emulsions. Emulsions were subsequently mixed by slowly rotating the container (Greiner Bio-one, Austria) and stored for 24 h at room temperature before further analysis.

2.2.3. Transglutaminase-induced clustering of o/w emulsions

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

room temperature on ice. The emulsion was stored for 24 h at room temperature before further analysis.

2.3. Physical characterisation of o/w emulsions

2.3.1. Determination of particle size and effective cluster size

The size of oil droplets in single emulsions was determined by static light scattering (Master sizer 2000S, Malvern Instruments Ltd., UK) and microscopy. The refractive index of the oil droplets was set as 1.45 and that of the dispersant at 1.33. Pump speed was set at 500 rpm. Droplet size was reported as d0.5, D[4,3] and D[3,2]. Each measurement was performed in triplicate. For size measurements by microscopy, emulsions were diluted with their corresponding aqueous phase (without emulsifier), placed on a microscope slide, and covered with a coverslip. Each sample was analysed at six positions using an optical light microscope (Axioskop 2 plus, Carl Zeiss AG, Germany) equipped with a camera (Axiocam ERc 5S, Carl Zeiss AG, Germany) and Visio imaging software. Recorded images were analysed for particle size (area) using ImageJ. To scale from pixel to µm, the scale of a microscopic picture (based on the built-in ZEN imaging software of the Axioskop 2 plus) was used. The brightness was adjusted automatically and a threshold of $<$ 0.2 µm was applied to remove background noise. An effective cluster size was calculated from the droplet area assuming a spherical shape. At least three independent emulsion replicates were used, probed at six positions, and an average diameter with standard deviation calculated and reported. Clustered emulsions cross-linked by proanthocyanidins and transglutaminase were also analysed for cluster size using static light scattering (Malvern Mastersizer 2000S, Malvern Instruments Ltd., UK). For the o/w emulsions clustered using charge-based clustering, clusters disintegrated during the static light scattering measurements due to insufficiently strong interactions. Therefore, cluster size could not be determined by light scattering and was assessed by image analysis as described before. The cluster size was reported as effective cluster size with standard deviation.

2.3.2. Determination of ζ -potential

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

2.3.3. Stability of clustered o/w emulsions at different pH and ionic strength

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

2.3.4. Rheological characterisation

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

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

2.4. Estimation of effective oil volume fraction of clustered o/w emulsions

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

$$\frac{\eta_{eff}}{\eta_0} = n_r = \left(1 - \frac{\phi}{\phi_m}\right)^{-[\eta]\phi_m} \quad (1)$$

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

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

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

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

3. Results and discussion

3.1. Oil droplet clusters of WPI-stabilised o/w emulsions with proanthocyanidins

By using the grape seed extract (GSE) Vitaflavan® as a proanthocyanidin source at pH 3, concentration-dependent clustering of oil droplets of WPI-stabilised o/w emulsions was observed (Fig. 1). Cluster size strongly increased with increasing GSE concentration. Starting with a single-droplet emulsion with oil droplets of around 1 µm diameter, the obtained cluster sizes ranged from 14 µm for 25% grape seed extract per gram whey protein to 140 µm for 75% grape seed extract per gram whey protein. 25% grape seed extract per gram whey protein was found

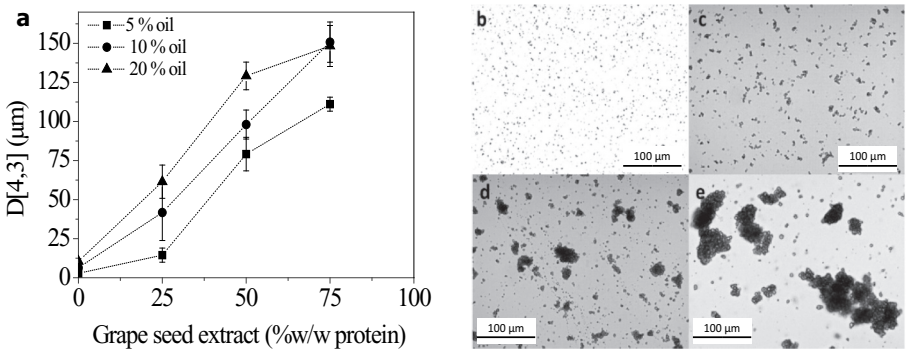

Fig. 1. a) Oil droplet cluster size (D(4,3)) as a function of grape seed extract concentration for WPI-stabilised o/w emulsions varying in oil content. Lines are added to guide the eye (n = 3, error bars indicate standard deviation). Right images: Micrographs of emulsions. The images show a non-clustered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

to be the minimum concentration required to cluster oil droplets.

The oil volume fraction of the emulsion affected cluster size in a non-systematic manner. For an increase in oil concentration from 5% to 20%, an increase in cluster size was observed. A decrease in distance between droplets by increasing volume fraction led to the formation of larger clusters. The formation of clusters and the efficiency in droplet clustering are a result of a variety of interactions occurring. It is known that proanthocyanidins present in grape seed extract vary in composition from mono- to heptametrical oligomers (Bohin et al., 2012). Due to the large diversity in oligomers, a variety of physical and chemical interactions can occur, leading to the formation of hydrogen bridges as well as covalent bonds. Bohin et al. (2012, 2014) argued that complexation of β-lactoglobulin with proanthocyanidins is based on an extended hydrogen bond network. We suggest that such interactions are also responsible for the clustering of WPI-stabilised o/w emulsion droplets by proanthocyanidins present in grape seed extract since WPI contains β-lactoglobulin as the main constituent. Additionally, the presence of proanthocyanidins has been suggested to alter the unfolding of globular proteins leading to exposure of hydrophobic side groups (Kanakis et al., 2011). Hydrophobic interactions and entanglements of unfolded protein strands increase the interactions and network formation between protein molecules, thus further stabilizing clusters. Next to hydrogen bonds and hydrophobic interactions, also stacking of polyphenolic rings through π-π interactions has been suggested as a possible contribution to the complexation (Charlton et al., 2002; Kanakis et al., 2011). These oil droplet clusters are therefore most likely stabilised by a variety of interactions.

Fig. 2 shows the influence of incubation time on cluster size of WPI-stabilised o/w emulsions with 20% oil, clustered with proanthocyanidins. Incubation time was varied from 4 to 72 h. It can be seen that the largest increase in cluster size was observed within the first 24 h. For a relatively low GSE content (25%, light grey bars), the cluster size increased to 20 µm after 4 h and to 60 µm after 24 h. For higher GSE concentrations (medium and dark grey bars), an increase in time from 4 to 24 h led to an increase in cluster size from 65 to 130 µm for 50% GSE per g protein, and from 100 to 165 µm for 75% GSE. However, at an additional increase in incubation time from 24 to 72 h, cluster size levelled off for higher concentrations of GSE and for low concentrations even seemed to decrease slightly. Consequently, the incubation time for further experiments was kept at 24 h.

We evaluated the stability of oil droplet clusters, expressed as cluster size, against changes in pH and ionic strength within 24 h. We would like to add that we evaluated the stability of the clusters, rather than the stability of the emulsions itself. Due to the presence of clusters, creaming of the emulsions occurred, but this was reversible upon gentle shaking. The clusters themselves did not change in this process. We related the final cluster size to the initial size immediately after preparation of the clusters (cluster $size_{end}$/cluster $size_{initial}$). An overview of the changes in cluster size upon changes in pH and ionic strength can be found in Fig. 3. Upon both a decrease from pH 3 to 2 and an increase from pH 3 to 8, cluster size decreased. Clusters disintegrated partly into single droplets, indicating a decrease of attractive interactions between the droplets. Cluster size strongly decreased between pH 3 and 5, which is close to the isoelectric point of the proteins. One could expect that the reduced positive charges of the proteins, due to a pH close to the isoelectric point, facilitate clustering due to less electrostatic repulsion. The disintegration is therefore not only related to changes in the electrostatic interactions between proteins. A potential reason for the disintegration could be a reduced affinity between whey proteins and proanthocyanidins at higher pH. In the case proteins were still positively charged when the pH was further reduced from 3 to 2, the size also decreased. We attribute this to possible degradation of interflavan bonds, which are covalent bonds between subunits of the proanthocyanidin, or other pH-sensitive bonds (Vidal, Cartalade, Souquet, Fulcrand, & Cheynier, 2002). Hydrogen bonds and π-π interactions may

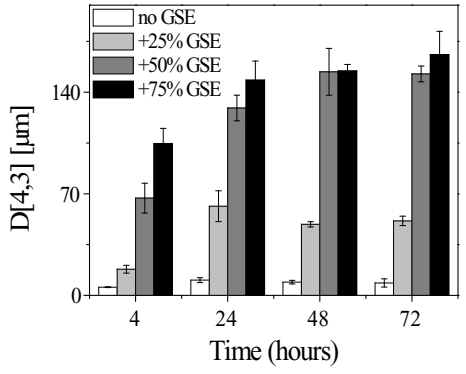

Fig. 2. Oil droplet cluster size (D4,3) as a function of incubation time at room temperature for WPI-stabilised o/w emulsions at 20% oil concentration (n = 3, error bars indicate standard deviation).

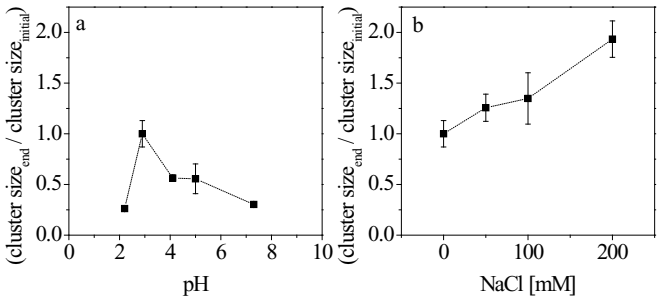

Fig. 3. Relative change in effective diameter (cluster $size_{end}$/cluster $size_{initial}$) of clustered emulsions as a function of changes in pH (a) and NaCl concentration (b). Dotted lines are added to guide the eye (n = 3, error bars indicate standard deviation).

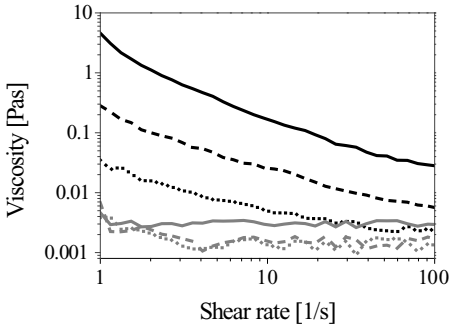

Fig. 4. Exemplary flow curves of non-clustered (grey) and clustered (75% GSE, black) o/w emulsions varying in oil concentration (5% oil: dotted, 10% oil: dashed and 20% oil: full line).

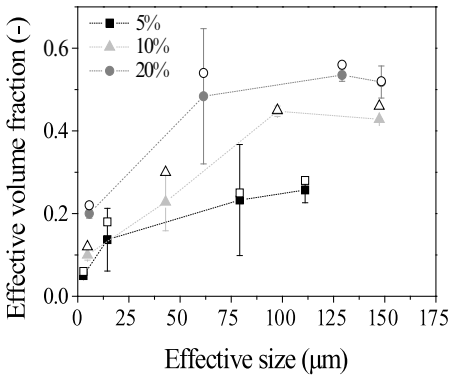

Fig. 5. Effective volume fraction of WPI-stabilised o/w emulsion clustered with grape seed extract (25%, 50% and 75% GSE per g protein) as a function of cluster size for oil volume fractions of 0.05, 0.10 and 0.20 (n = 3, error bars are standard deviation). Closed symbols: estimates of effective volume fraction obtained with Krieger-Dougherty model; open symbols: estimates of effective volume fraction obtained from an "individual droplet calibration curve".

also change with changing pH (Ozdal, Capanoglu, & Altay, 2013). These results suggest that the clusters formed by clustering of WPI-stabilised o/w emulsion droplets with proanthocyanidins are mainly stabilised by non-covalent interactions, as evidence for covalent interactions could not be found.

Cluster size also varied upon changes in ionic strength. Cluster size increased linearly with increasing NaCl concentration from a diameter D[4,3] of 60 µm at standard buffer conditions (no added NaCl) to 100 µm at 200 mM of added NaCl. This was most likely related to a reduction of electrostatic repulsion between the WPI-stabilised o/w emulsion droplet clusters at higher ionic strengths, which allowed further growth of the clusters. These results show that electrostatic interactions are of importance both within the cluster and between clusters. The clusters showed sensitivity to changes in pH, by cluster breakdown, and sensitivity to changes in the ionic strength; cluster size increased with increasing ionic strength.

The effect of droplet clustering on the rheological properties of the emulsions was examined. In Fig. 4, examples of flow curves of non-clustered and clustered (75% GSE) o/w emulsions varying in oil concentration (5, 10 and 20%) are presented. Single oil droplet emulsions showed Newtonian flow behaviour, with a viscosity of around 0.001 to 0.01 Pa·s. Upon clustering of the WPI-stabilised emulsions (5, 10 and 20% oil concentration) viscosity increased and displayed strong shear thinning behaviour. For the largest clusters stabilised with 75% GSE (*w*/w protein) and with an oil concentration of 20%, the viscosity increased by at least a factor 100 at low shear rates to values > 1 Pa·s. The increase in viscosity was an effect of the increase of the effective oil volume fraction as a result of the oil droplet clustering (Montesi, Peña, & Pasquali, 2004; Starov & Zhdanov, 2003). The larger the oil droplet clusters at a certain absolute oil volume fraction, the higher the effective volume fraction of the droplets due to entrapment of the bulk liquid between oil droplets within the clusters. Wu and co-workers showed comparable findings, in terms of rheological behaviour, in clustered emulsions using divalent salts, indicating an increasing viscosity with increasing cluster size. (Wu, Degner, & McClements, 2013) Furthermore, the presented results are in line with studies on non-controlled, depletion-induced clustering of droplets, where a considerable increase in viscosity and shear thinning behaviour were reported. (Berli, Quemada, & Parker, 2002; Dickinson & Golding, 1997).

In Fig. 5, the effective volume fraction of clustered o/w emulsions, estimated roughly using the Krieger-Dougherty model (Eq. (1)), is shown as a function of the effective size of droplet clusters (filled symbols). With increasing cluster size, the amount of entrapped continuous phase was enhanced, and the effective volume fraction increased. It can be seen that for a 0.05 oil volume fraction (5%, squares), the effective volume fraction increased to 0.25. In the case of an oil volume fraction of 0.20 and for the largest cluster sizes, the effective oil volume fraction of the clustered WPI-stabilised emulsions increased to values of around 0.55. We emphasize again that the estimated effective volume fraction of the clustered emulsions obtained using the Krieger-Dougherty equation may only be a crude estimate as assumptions regarding droplet shape and droplet size distribution are violated. A better estimate of the intrinsic viscosity and including the droplet polydispersity could be improvements in this approach. However, these aspects are out of the scope of this study.

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

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

The critical strain and the stress at critical strain were used to estimate the apparent interaction strength or cluster strength (Mancini, Montanari, Peressini, & Fantozzi, 2002). Examples for amplitude sweeps can be found in the supplementary materials. For emulsions with an oil volume fraction of 0.20, critical strain values of 1.37 ± 0.40% for 25% GSE, 4.30 ± 2.05% for 50% GSE and 6.43 ± 1.30% for 75% GSE were found. The stress at critical strain increased with increasing GSE concentration from 0.2 Pa for 25% GSE to 2.0 Pa for 50% GSE and 8.8 Pa for 75% GSE. We suggest that not only the effective size of the cluster increases with increasing concentration of grape seed extract, but also the strength of the clusters. Above a certain total oil volume fraction and GSE concentration, a space spanning network is formed.

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

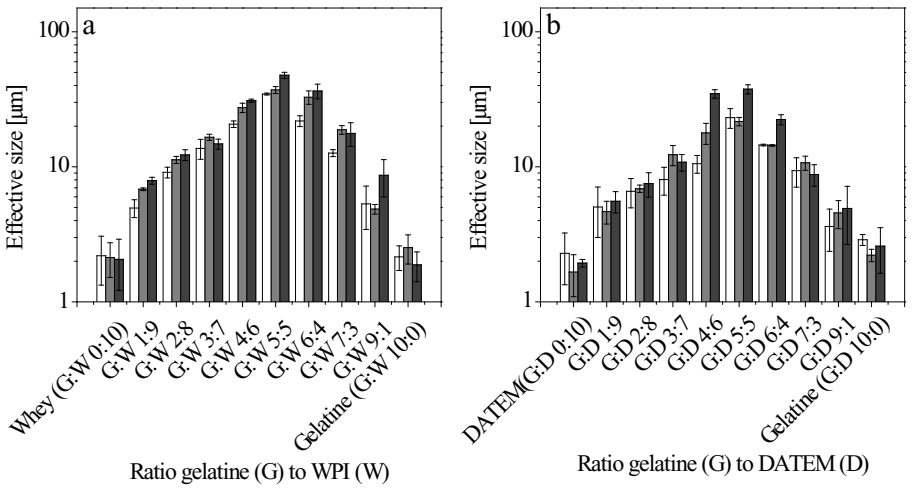

Fig. 6. Effective size of hetero-aggregated o/w emulsions as a function of mixing ratio of oppositely-charged o/w emulsions. a) Hetero-aggregates prepared by mixing gelatine and WPI-stabilised emulsions at pH 7. b) Hetero-aggregates prepared by mixing gelatine- and DATEM-stabilised emulsions at pH 5. Effective diameter is shown as function of oil concentration at 5% (white), 10% (grey) and 20% (black) (all *v*/v). (n = 3, error bars indicate standard deviation).

3.2. Hetero-aggregation of o/w emulsions

To obtain clusters stabilised by charge-based interactions, emulsion droplets with an initial size of around 2 µm were clustered with hetero-aggregation. Two sets of emulsions with oil droplets stabilised with oppositely-charged emulsifiers were used. The emulsifier combinations were (1) gelatine (pI = 8–9) and WPI (pI = 4.5) at pH 7 and (2) the novel combination gelatine and DATEM (pKa = 3) at pH 5. DATEM-stabilised emulsions were negatively charged, with a constant zeta potential of around −60 mV over a pH range from 3 to 7. WPI-stabilised emulsions displayed an average zeta potential of 0 mV at pH 5, close to the pI of WPI, a positive charge (+25 mV at pH 3) below the pI and a negative charge (−48 mV at pH 7) above the pI. Gelatine (pI around 8–9) showed a slightly positive charge of +10 mV at pH 5 and of +7 mV at pH 7. To obtain hetero-aggregated emulsions with a large zeta-potential difference (Δζ), gelatine emulsions were used as a source for the positively- charged oil droplets. These droplets were mixed with either negatively-charged WPI-stabilised droplets at pH 7, or DATEM-stabilised droplets at pH 5. When combining the different single emulsions in various mixing ratios, a broad range of cluster sizes was obtained (Fig. 6). The mixing ratio strongly affected the effective cluster size of clustered emulsions. A large increase in cluster size with increasing oil volume fraction was observed for gelatine-WPI hetero-aggregated emulsions (Fig. 6a), whereas a smaller increase was observed for the gelatine-DATEM hetero-aggregated emulsions (Fig. 6b). Both emulsions with hetero-aggregated clusters exhibited a maximum cluster size at a mixing ratio of 5:5. For the gelatine-WPI hetero-aggregated emulsion with an oil volume fraction of 0.2 at a ratio 5:5, cluster size was around 50 µm (Fig. 6a), which represented a large increase from the single droplet diameter of 2 µm. The corresponding gelatine-DATEM emulsion displayed a cluster size of around 40 µm (Fig. 6b). Cluster size tended to increase to a small extent with increasing oil volume fraction. The dependency of cluster size on mixing ratio can be explained based on the charge stoichiometry; an equal quantity of positively- and negatively-charged droplets of the same size is necessary to create the largest possible clusters. In presence of an excess of one of the charged droplets, the cluster growth is limited by the droplet type that is less abundant. These findings are in agreement with previous studies using different oppositely charged emulsifiers, in which the largest cluster size was found towards intermediate mixing ratio of hetero-aggregated o/w emulsions (Maier et al., 2015; Maier, Ensenberger, Irmscher, & Weiss, 2016; Mao & McClements, 2011, 2012a; Simo, Mao, Tokle, Decker, & McClements, 2012).

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

However, as shown in Fig. 7, gelatine-WPI clusters (Fig. 7a) were more open and appeared larger than clusters prepared with gelatine and DATEM (Fig. 7b), which were smaller, and more compact and spherical. Even though the interactions among droplets in gelatine-DATEM clusters are stronger, they are apparently not strong enough to

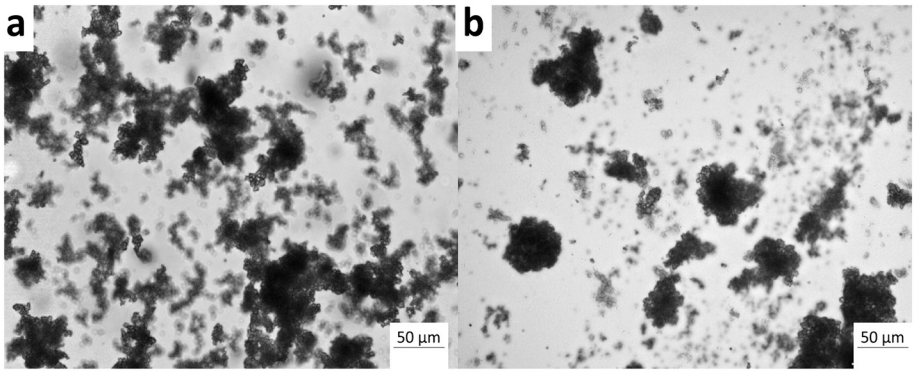

Fig. 7. Micrographs (light microscopy) of two hetero-aggregated o/w emulsions; a) gelatine-WPI hetero-aggregates (5:5 mixing ratio) and b) gelatine-DATEM hetero-aggregates (5:5 mixing ratio).

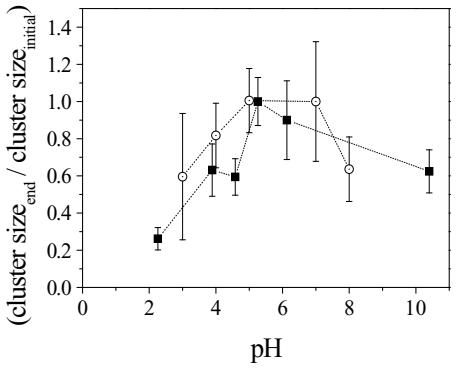

Fig. 8. Relative change in effective diameter (cluster size$_{end}$/cluster size$_{initial}$) of clustered emulsions as a function of changes in pH. Gelatine-DATEM hetero-aggregated o/w emulsions (5:5 mixing ratio) are depicted in black squares and gelatine-WPI hetero-aggregated o/w emulsions (5:5 mixing ratio) in open circles. Dotted lines are added to guide the eye (n = 3, error bars indicate standard deviation).

prevent rearrangements. These stronger interactions with possible rearrangements seem to lead to more compact aggregates. Mainly, we suggest that the open and more "cloudy" structures of the gelatine-WPI clusters are an effect of their very weak interactions.

To test the stability of hetero-aggregated clusters against environmental conditions, the ionic strength was varied from 3 mM to 180 mM NaCl and the pH from 3 to 8. Stability was assessed by investigating changes in cluster size by relating the final cluster size to the initial size immediately after preparation of the clusters (cluster size$_{end}$/cluster size$_{initial}$). A change of pH in the hetero-aggregated emulsion had a large effect on the stability of clusters (Fig. 8). For both cluster types, the cluster size was largest at the pH of preparation. Gelatine-WPI hetero-aggregated emulsions were prepared at pH 7, and at this pH the clusters showed the highest stability. When pH was decreased slightly to pH 5, no differences in cluster size were seen yet and clusters appeared to be stable. However, when the pH was further decreased to pH 3 or increased to pH 8, clusters became more unstable and the effective diameter decreased to 60% of the initial size. Similarly, for the DATEM-gelatine clusters prepared at pH 5, clusters were observed to be most stable at this pH. Cluster size decreased to 20% of their initial cluster size when pH was either increased to 11 or decreased to pH 2. At both low and high pH, gelatine-WPI and gelatine-DATEM hetero-aggregated emulsions decreased in cluster size due to a decrease in electrostatic attraction. In case of gelatine-WPI hetero-aggregated emulsions, at low pH both gelatine and WPI carry a positive charge, leading to electrostatic repulsion, which initiates the disintegration of the clusters. At high pH, gelatine has either a net zero charge or slightly negative charge, and electrostatic interactions are limited. Both DATEM and WPI are negatively charged at high pH, thus clusters can disintegrate through electrostatic repulsion. This behaviour indicates the high importance of electrostatic forces in the stabilisation of the hetero-aggregated clusters.

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

The clustering of the droplets had a large influence on the rheological properties of the emulsions. Representative flow curves can be found in Fig. 9 showing o/w emulsions with non-clustered and highly clustered emulsions at the tested oil concentrations. Gelatine-stabilised, DATEM-stabilised and WPI-stabilised single-droplet emulsions showed Newtonian flow-behaviour with viscosities ranging from 0.001 to 0.01 Pa·s at volume fractions of 0.05–0.20. Upon clustering the emulsions by hetero-aggregation, the viscosity increased up to about three orders of magnitude, depending on cluster size and cluster type and shear thinning flow behaviour was introduced. Viscosity increased to about 3 Pa·s for highly clustered emulsions of gelatine-DATEM and 1 Pa·s for gelatine-WPI emulsions at low shear rates (1–10/s). Reasons for the increase in viscosity have already been discussed previously for grape seed extract clustered emulsions. In this case, we saw that viscosity is not only determined by cluster size, but that the cluster properties also play an important role. Even though the gelatine-DATEM clusters were smaller, the viscosity increase was the largest, which indicates that the cluster strength also plays an important role. When comparing our data with the research of Mao and McClements (2012b, 2013), the results are in good agreement. The viscosity values measured for hetero-aggregated emulsions in our study were higher than those in the hetero-aggregated systems consisting of modified starch-whey protein-stabilised clusters (Mao & McClements, 2013), but comparable to the viscosity values of clusters stabilised by lactoferrin and beta-lactoglobulin (Mao & McClements, 2012b). We conclude that cluster size and interaction strength, derived from emulsifier charge and –type, determine rheological properties of clustered emulsions at a given oil volume fraction.

Fig. 10 presents the effective volume fraction estimated using the Krieger-Dougherty model as a function of cluster size for both hetero-aggregated emulsions (gelatine-DATEM and gelatine-WPI) at 0.20 oil volume fraction. With an increasing cluster size, the effective volume fraction increased for both emulsion types. The effective volume fraction increased to about 0.50 for both emulsions, which is 2.5 times larger than the absolute oil volume fraction of 0.20. Similarly, for emulsions with 0.10 oil volume fraction, the effective volume fraction increased to up to 0.50, and thus increased by a factor of 5.

As previously discussed, the rheological properties of the clustered emulsions depend on the properties of the clusters. The cluster

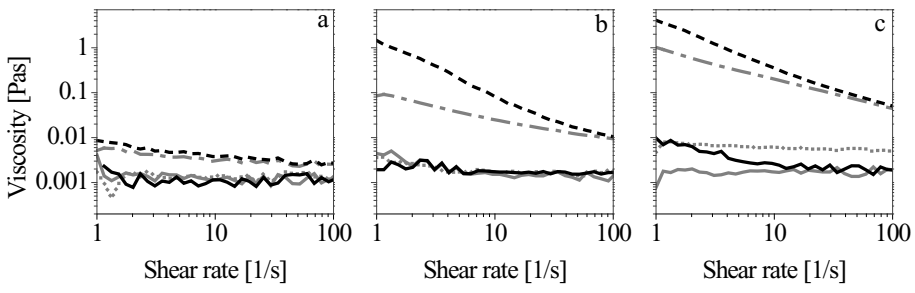

Fig. 9. Flow curves of non-clustered and hetero-aggregated o/w emulsions varying in oil concentration (a: 5%, b: 10%, c: 20%). Black line shows DATEM-stabilised emulsions, dotted line shows gelatine-stabilised emulsion, grey line indicates whey-stabilised emulsions, dashed-dotted line depicts large clusters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

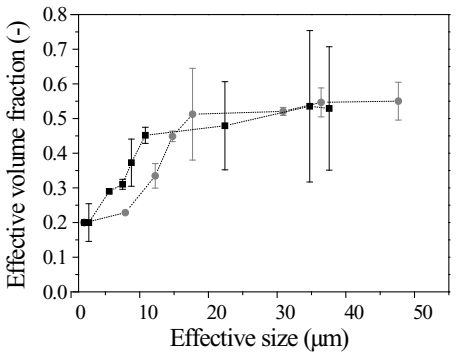

Fig. 10. Effective oil volume fraction as a function of oil droplet cluster size of hetero-aggregated o/w emulsions. Black squares represent gelatine-DATEM hetero-aggregated o/w emulsion with 0.20 oil volume fraction and grey circles represent gelatine-WPI hetero-aggregated emulsions with 0.20 oil volume fraction. Lines are added to guide the eye (n = 3, error bars indicate standard deviation).

interaction strength is determined by the strength of the electrostatic interactions. Upon shearing, clusters may be torn apart when the shear force applied is larger than the force holding the cluster together, thereby decreasing the viscosity. Furthermore, cluster-cluster interactions have to be taken into consideration. With increasing total oil-volume fraction, the degree of free movement of the clusters are reduced, which is expected to increase the viscosity. We measured the elastic modulus, G', and estimated the critical strain of the hetero-aggregated emulsions to gain insight into the interaction strength within and between clusters. Emulsions with small clusters, e.g. in a mixing ratio 1:9 and 2:8, showed mainly viscous behaviour and the elastic modulus upon increasing strain was insufficiently stable to determine a critical strain. Larger clusters at the same oil volume fraction, e.g. with ratios of 4:6, 5:5 and 6:4, showed comparable critical strains. As an example for the difference in cluster type, we discuss the results for clusters of a mixing ratio 4:6. Gelatine-WPI hetero-aggregated emulsions had a critical strain of 1.77 ± 0.60% (and stress at a critical strain of 2 Pa). In comparison, gelatine-DATEM emulsions with the same ratio exhibited a much larger critical strain of 4.12 ± 0.80 and a stress at a critical strain of 6 Pa (examples of measurements can be found in the supplementary material). Based on these values, more and stronger interactions are assumed to be present in gelatine-DATEM hetero-aggregated emulsions than in gelatine-WPI hetero-aggregated emulsions. This is again consistent with the larger difference in charge density between oil droplets, as determined by zeta-potential measurements.

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

3.3. Clustering of oil droplets using transglutaminase

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

3.4. Comparison of hetero-aggregation and clustering with grape seed extract

In this study, we aimed at obtaining oil droplet clusters varying both in cluster size and cluster strength to adjust the rheological properties of emulsions. An overview of properties of selected samples can be found in Table 2. We observed that clustering of WPI-stabilised emulsions with proanthocyanidins and hetero-aggregation of oppositely-charged emulsions allowed to control clustering oil droplets in o/w emulsions in a large size range. In Table 2 it can be seen that cluster size could be varied between a few microns to 50 μm for hetero-aggregation and up to 150 μm for clustering with GSE. Cluster size and the resulting effective volume fraction were shown to depend on the approach used. Fig. 11 shows the effective volume fraction as a function of cluster size for the two clustering methodologies. For the same cluster size, the effective oil volume fraction was smaller for the emulsions cross-linked with grape seed extract (proanthocyanidins) than for the hetero-aggregated o/w emulsions. We suggest that the effective oil volume fraction is related, to a certain extent, to the interaction strength of the clusters. The interaction strength within the clusters was shown to depend on the interactions responsible for the clustering. Large clusters prepared with grape-seed-extract (75% GSE/protein) showed a critical strain of 6.43 ± 1.30%, whereas the largest clusters (about 50 μm) of strongly bound gelatine-DATEM emulsions showed a lower critical strain of about 4.0 ± 0.8%. The multiple interactions in the grape seed extract stabilised droplet clusters lead to stronger clusters than the electrostatic interactions in the hetero-aggregates. As a consequence, the packing of the oil droplets within the cluster and the ability to entrap the continuous phase are affected; stronger interactions cause a more close droplet packing and less water will be entrapped, leading to a lower effective volume fraction. Accordingly, the effective oil volume fraction for loosely clustered emulsions (hetero-aggregation) is higher. The differences in effective oil volume fraction between the two sets of hetero-aggregated emulsions are not discussed here due to rather large standard deviations of the measurements. As consequence of the clustering and the increase in effective volume fraction, for both methodologies, the viscosity increased. In Table 2, the viscosities at a shear rate of 50/s are shown. As expected, viscosity increases with increasing clustering of the droplets. Based on these results, we conclude that the presented methods of clustering allow not only a control in cluster size, but also a control in interaction strength and cluster morphology, which provides a tool to alter the rheological behaviour of the resulting emulsions.

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

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

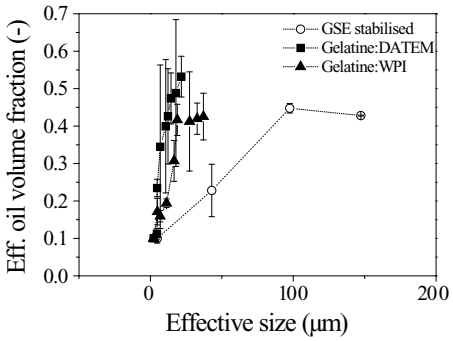

Fig. 11. Effective volume fraction of o/w emulsions as a function of the degree of oil droplet clustering for the different clustering methodologies: hetero-aggregation (full symbols: squares are gelatine-DATEM hetero-aggregated o/w emulsions, triangles are gelatine-WPI hetero-aggregated o/w emulsions) and open circles are WPI-stabilised o/w emulsions cross-linked with grape seed extract (proanthocyanidin). Absolute oil volume fraction is 0.10 (oil concentration 10%) for all emulsions. Error bars are standard deviations (n = 3) and dotted lines are added to guide the eye.

4. Conclusions

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

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Competing interest

The authors have declared that no competing interests exist.

Financial disclosure

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.04.027.

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