

Bulk and interfacial properties of milk fat emulsions stabilized by whey protein isolate and whey protein aggregates

Xilong Zhou, Guido Sala, Leonard M.C. Sagis^{*}

Physics and Physical Chemistry of Foods, Wageningen University, Bornse Weiland 9, 6708WG, Wageningen, the Netherlands

ARTICLE INFO

Keywords:

Recombined dairy cream
Whey protein (aggregate)
Bulk stability
Interfacial rheology

ABSTRACT

Whey protein is widely used in the food industry as an emulsion stabilizer because of its outstanding emulsifying ability. Recent studies have shown that heat-induced whey protein aggregates may also have potential to stabilize emulsions. The interfacial behavior of whey protein and whey protein aggregates adsorbed at the milk fat-water interface has not been well investigated, especially not in the nonlinear regime, which is highly relevant for the preparation of products such as recombined dairy cream.

In this study, the interfacial properties of milk fat-water interfaces stabilized by whey protein isolate (WPI) and whey protein aggregates (WPA) at different bulk concentrations (0.1 wt% - 4.0 wt%) were studied by Large Amplitude Oscillatory Dilatation (LAOD). Lissajous plots were used to analyse the nonlinear response of the interfaces as a function of strain amplitude and frequency. The elastic modulus was quantified based on the tangent modulus at zero instantaneous strain in expansion and in compression. Bulk stability of creams stabilized with the mentioned proteins was studied by determining creaming rate, droplet size distribution, ζ -potential and viscosity of the continuous phase.

At low concentrations (<2.0 wt%), WPI-stabilized cream had smaller oil droplets than WPA-stabilized cream, indicating that at these concentrations WPI had better emulsifying ability. For concentrations higher than 2.0 wt%, WPA was a better emulsifier in terms of creaming stability because of the higher viscosity of the continuous phase of the emulsions. Both WPI and WPA could prevent coalescence equally well if the concentration was higher than 0.5 wt%. LAOD measurements showed that at a protein concentration of 0.1 wt%, there was little difference between WPI- and WPA-stabilized interfaces. At 4.0 wt%, WPI showed abrupt intra-cycle yielding followed by a predominantly viscous behavior at large expansion. The WPA interfacial layer had a larger maximum linear strain, and showed a more gradual softening in expansion and mild strain hardening in compression. We hypothesize that WPI formed denser and more brittle (quasi-) 2d structures at the interface, while the interfaces formed by WPA might have a thicker and more stretchable 3d structure. The WPA-stabilized emulsion was less resistant to coalescence upon drastic stirring, which can be explained with its different large deformation behavior, and is relevant for applications where the cream is subjected to large deformations (whipping or stirring).

1. Introduction

Kinetic stability of oil-in-water (o/w) emulsions has been a topic of considerable interest for a long time. A frequent aim of studies on this subject is to link aspects like creaming rate, droplet size distribution and viscosity of the continuous phase to the composition of the continuous phase and/or properties of the interface between the two immiscible phases. Low molecular weight surfactants or proteins are the most frequently used stabilizers for food emulsions. For small surfactants, the

Gibbs-Marangoni effect is the main mechanism of stabilization of an interface (Tadros, Izquierdo, Esquena, & Solans, 2004). Proteins adsorb at the interface more slowly than low molecular weight surfactants because of their larger molecular size and complex structure. After emulsion formation, proteins form viscoelastic interfacial layers that, apart from mechanical stabilization, can provide steric and electrostatic repulsion among emulsions droplets. This way, several instability phenomena could be retarded (Damodaran, 2005; Dickinson, 1999; Dickinson, 2001; Wilde, 2000). Different proteins contribute differently to

^{*} Corresponding author.

E-mail address: leonard.sagis@wur.nl (L.M.C. Sagis).

<https://doi.org/10.1016/j.foodhyd.2020.106100>

Received 28 February 2020; Received in revised form 20 May 2020; Accepted 10 June 2020

Available online 16 June 2020

0268-005X/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

emulsion properties, mainly because of differences in the interfacial structures they form and in the interfacial composition (Dalglish, 2006).

Milk fat based emulsions, such as recombined dairy cream and recombined milk, are a group of emulsions of increasing economic interest. In the production of these emulsions, anhydrous milk fat is mixed with a solution of non-fat milk solids, and subsequently homogenized. Compared with other emulsions, milk fat based emulsions have some potential differences, which mainly result from the complicated chemical composition of milk fat. Milk fat has a wide variety of triglycerides containing fatty acids with varying levels of saturation, and bimodally distributed in terms of carbon numbers (Yener & van Valenberg, 2019). Because of the presence of short-chain fatty acids in the triglycerides, but also of aldehydes, ketones and lactones, milk fat has a unique flavor and cannot easily be replaced by other animal fats or plant oils (Mortensen, 2016).

In contrast to milk fat globules in natural milk, which have substantial long-time stability imparted by the milk fat globule membranes, the milk fat droplets in recombined dairy cream tend to be much less stable, and additional stabilizers need to be added to improve emulsion stability. Several stabilizers have been proposed for this purpose, such as proteose-peptone, glycerol monostearate, tween 80, lecithin and phospholipid-enriched dairy products. Most of the studies on the effect of the mentioned components in recombined milk fat emulsions focused on whipping properties (Fredrick et al., 2013; Han et al., 2018; Phan, Moens, Le, Van der Meeren, & Dewettinck, 2014; van Lent, Le, Vanlerberghe, & Van der Meeren, 2008; Vanderghem, Danthine, Blecker, & Deroanne, 2007) and little on the macroscopic stability of the system as a function of composition (Fredrick et al., 2013; Vanderghem et al., 2007; S.; Wu et al., 2016; Zhou et al., 2016). Studies on milk fat-water interfaces are still scarce, in spite of the important role of interfacial properties in milk fat emulsion stability, both under quiescent conditions and during whipping.

Whey protein is widely used in the food industry, not only because it contains all essential amino acids and is well digestible (Hoffman & Falvo, 2004), but also because of its good emulsifying ability. The physicochemical properties of whey protein and its applications in emulsion are clearly discussed in several reviews (Damodaran, 2005; Nicolai, Britten, & Schmitt, 2011). Many studies have been devoted to the interfacial properties of whey protein-stabilized interfaces (Davis & Foegeding, 2004; Petkov, Gurkov, Campbell, & Borwankar, 2000; Rodríguez Patino, Rodríguez Niño, & Sánchez, 1999; Wooster & Augustin, 2007). Most of these either focused on air-water interfaces or on plant oil-water interfaces, and very few studies are available on the interfacial properties in the large deformation (i.e. nonlinear response) regime. However, during manufacturing (e.g. homogenization, pumping, whipping) the droplets in emulsions are routinely subjected to large deformations. Consequently, studying the interfacial properties of oil-water interfaces at large and fast dynamic strains is extremely relevant, and for the dynamic stability of emulsions, it is surely more relevant than small amplitude oscillatory deformations at low frequencies. Lissajous plots are a powerful tool to study the interfacial properties of protein-stabilized interfaces in the nonlinear regime and have already been utilized in several studies. Schröder, Berton-Carabin, Venema, and Cornacchia (2017); Wan, Yang, and Sagis (2016); Chen et al. (2017) have studied the interfacial properties of interfaces stabilized by whey protein hydrolysates, soy protein fibrils and casein micelles, respectively, using large amplitude oscillatory dilatations. The anhydrous milk fat-water interface has so far not been studied with this approach.

In recent decades, several studies have appeared in which the effects of modifications of native whey proteins, such as (partial) hydrolyzation, enzymatic modification, or heat induced aggregation, on the emulsifying properties, were investigated. Some studies claim that after heating between 60 and 90 °C, whey protein will lose its emulsifying ability to a great extent (Dybowska, 2011; Millqvist-Fureby, Elofsson, & Bergenstahl, 2001). However, according to Dybowska (2011); Nicolai

and Durand (2013), whey protein aggregates prepared by controlled heat treatment can improve emulsion stability. Dybowska (2011) hypothesized that the improved stability is the result of the formation of thicker interfacial layers around the oil droplets. This opinion is supported by transmission electron micrographs made by Foley and O'Connell (1990). In summary, although the emulsifying ability of whey protein aggregates is worse than that of native whey protein, aggregates can apparently still stabilize emulsions, mainly because of the interfacial structures they form. The mechanical properties of these interfacial structures have not been well studied. Recently, the application of whey protein microgel particles in emulsion stabilization has also attracted much attention. Microgels are claimed to adsorb at the interface to form Pickering emulsions, and efficiently prevent coalescence. Whey protein microgels are made without cross-linking agents (Schmitt et al., 2010), and are promising materials to encapsulate emulsions to delay lipid digestion (Sarkar et al., 2016) or for drug delivery (Jiang, Chen, Deng, Suuronen, & Zhong, 2014). A few studies have investigated the effects of pH, ionic strength, protein concentration on the stability of emulsions stabilized by whey protein microgels and on the microstructure of the interfaces (Destribats, Rouvet, Gehin-Delval, Schmitt, & Binks, 2014; J.; Wu et al., 2015). Again, the mechanical properties of these structures at the interface have not been investigated in detail. In particular, data in the nonlinear response regime are lacking, and the relation between (nonlinear) interfacial properties and emulsion stability under quiescent conditions and far from equilibrium conditions still needs to be explored. Considering the application of plant protein is quickly gaining interest nowadays, an accurate characterization of the properties of dairy ingredients provides benchmarks for future studies on non-dairy materials.

The aim of the present study was to investigate the interfacial properties of anhydrous milk fat-water interfaces stabilized by native whey protein (WPI) and heat-induced aggregates (WPA), and link these properties and bulk properties of the continuous phase to the stability of model milk fat emulsions at quiescent and dynamic conditions. The interfacial properties of anhydrous oil-water interfaces were studied by large amplitude oscillatory dilatation (LAOD). Lissajous plots were utilized to characterize the nonlinear response of WPI- and WPA-stabilized interfaces at different bulk concentrations (0.1 wt% and 4.0 wt%). The bulk stability of the milk fat emulsion was studied in terms of droplet size distribution, viscosity of the continuous phase, creaming rate, and ζ -potential. The stability of the milk fat emulsions in dynamic conditions was also investigated by subjecting the emulsions to vigorous stirring, and monitoring the effects of this processing step on emulsion stability.

2. Materials and methods

2.1. Materials

Anhydrous milk fat was kindly donated by FrieslandCampina (Wageningen, Netherlands). Whey protein isolate (WPI, 88.8% protein content) was purchased from Davisco (USA). According to the specification sheet provided by Davisco, the lactose and calcium content of WPI were 0.2 wt% and 0.1 wt%, respectively. Florisil (60–100 mesh), 8-anilino-1-naphtalenesulfonic acid ammonium salt (ANS), sodium dodecyl sulfate (SDS) and sodium azide were purchased from Sigma (Netherlands). The phosphate buffer (PB, 0.01M, pH 7.0) used to measure protein hydrophobicity was made from sodium dihydrogen phosphate monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) and di-sodium hydrogen phosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) (Merck, Germany).

2.2. Methods

2.2.1. Sample preparation

2.2.1.1. Purification of anhydrous milk fat. Florisil was desiccated

overnight at 120 °C in an oven, then cooled down to room temperature. Anhydrous milk fat was melted at 60 °C and mixed with 10 wt% Florisil. The mixture was stirred while being heated in water bath set at 60 °C. Samples of anhydrous milk fat were taken every hour to measure the interfacial tension of a milk fat - Milli-Q water interface, until a constant value was obtained. Finally, Florisil was removed from the milk fat using filter papers (Whatman, Grade 4, diam.90 mm, England). The purified anhydrous milk fat was stored at −20 °C.

2.2.1.2. Whey protein solutions and whey protein aggregate (WPA) solutions. WPI powder was dissolved in Milli-Q water overnight to obtain a 6.25 wt% WPI solution; 0.02 wt% sodium azide was added to prevent spoilage. The WPI solution was centrifuged for 30 min at 10⁴ g to remove non-dissolved material (most likely insoluble aggregates). The supernatant was subsequently filtered by a syringe filter with pore size 0.45 µm and then stored as a WPI stock solution. The protein content of the stock solution was 5.49 wt%, as determined by Dumas (conversion coefficient: 6.25) and the pH was 6.8–7.0. Subsequently, the stock solution was diluted with MilliQ water to make samples with protein concentrations of 0.1, 0.2, 0.5, 1.0, 2.0 and 4.0 wt%.

The WPA aggregate dispersion was made from WPI stock solution. The stock solution was poured into a glass 250 mL beaker, and subjected to heat treatment in a water bath (80 °C, 30 min). During the heat treatment, the solution was stirred (300 rpm) with a magnetic stirrer (Framo, M20/1, Germany). The stirring bar had a length of 4 cm. Afterwards, the sample was cooled down by ice water, and diluted by Milli-Q water to obtain samples with protein concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, and 4.0 wt%.

2.2.1.3. Cream preparation. Cream samples with different stabilizer concentrations, both WPI and WPA, were prepared. The anhydrous milk fat was melted at 60 °C, then poured into the protein solutions to produce a mixture with 20 wt% fat. The mixture was kept at 60 °C in a water bath for 15 min. Subsequently, it was pre-homogenized using an Ultra-Turrax (IKA T25, Germany) at 6000 rpm, and finally homogenized by two-steps homogenization (Delta Instruments, Netherlands). Pressure was set at 100 bar for the first step and 40 bar for the second step. Samples were sealed in blue cap bottles and stored overnight at room temperature before analysis.

2.2.2. Characterization of the protein samples

2.2.2.1. Particle size distribution. The particle size distribution of WPI or WPA was determined using a Malvern Zetasizer Nano-ZS (Malvern Instruments Ltd, United Kingdom) at 20 °C, with cell type DTS0012. Protein solutions were filtered by syringe filter with pore size 0.45 µm and subsequently diluted to 0.4 wt% with Milli-Q water. The refractive and absorption indices were 1.450 and 0.001 respectively. The refractive index of dispersant (water) was 1.330. Before each test, samples were equilibrated for 2 min.

2.2.2.2. Hydrophobicity. Protein surface hydrophobicity was measured with methods described by Lam and Nickerson (2015). WPI solutions were diluted with PB buffer (0.01 M, pH 7.0) to obtain a concentration range from 0.02 wt% to 0.1 wt%. WPA solutions were diluted to a range from 0.005 wt% to 0.04 wt%. For each protein sample, 1 mL was added in a cuvette (10 × 10 × 45 mm, SARSTEDT, Germany) and mixed with 10 µL of 8 mmol/L 8-anilino-1-naphthalenesulfonic acid ammonium salt (ANS) solution (in 10 mM PB, pH 7.0). The mixture was incubated in the dark, while being shaken for 1 h. PB-ANSA without protein was used as a blank, and PB-protein was the control. Fluorescence was measured using a Fluorimeter (PerkinElmer, UK) at excitation wavelength of 390 nm, and emission wavelength of 470 nm. The slit width was set at 5 nm. The intensities of blank and control were subtracted for each protein sample to obtain the net fluorescence. The slope of the net fluorescence as the

function of protein concentration was used to quantify the extent of hydrophobicity. All measurements were made in triplicate.

2.2.3. Characterization of the milk fat emulsions

2.2.3.1. Creaming rate. A LUMiFuge (LUM LUMGmbH, Germany) was used to test the creaming behavior of samples at a constant gravitational acceleration value at room temperature (20 °C). The samples were centrifuged at 1000 g for 2600 s, which is equivalent to approximately one month of storage at 1 g. The light factor was set to 1.0. The measurement time interval was equal to 10 s. The creaming rate was calculated using the LUMiFuge Front Tracking module, and the tracked transmission value was 25%. The creaming rate is defined as:

$$\text{Creaming rate} = \frac{|\Delta L|}{\Delta t} \quad (1)$$

where ΔL is the change of position of the layer with 25% transmission in the time period Δt . Only the linear part of the curve of layer position versus time was considered.

In order to analyse the degree of coalescence or aggregation during centrifugation, samples were carefully taken from the tubes with a syringe, and their droplet size distribution was measured as described here below.

2.2.3.2. Oil droplet size distribution. The oil droplet size distribution of the emulsions was tested using a MasterSizer 2000 (Malvern Instruments Ltd., UK) with static light scattering. The cream was dispersed in distilled water until the obscuration was 15%. The refractive indices used for the dispersed phase (anhydrous milk fat) and dispersant were 1.461 and 1.330, respectively. The absorption index was 0.01. The weight-volume mean oil droplet diameter $d_{4,3}$ (µm) was calculated with Eq. (2).

$$d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (2)$$

where n_i is the number of particles with the same diameter, and d_i is the particle diameter.

For checking the degree of aggregation of droplets, the oil droplet size distribution of emulsion samples with added SDS was also tested. Samples were mixed in a 1:1 vol ratio with 1.0 wt% SDS solution, and then 100 times diluted by distilled water, before measuring the droplet size distribution.

2.2.3.3. Viscosity of continuous phase. The viscosity of continuous phase was tested with an Ubbelodhe capillary viscometer with constant 0.01078 mm²·s^{−2} (SI Analytics GmbH, Germany) at 20 °C. The dynamic viscosity η (mPa·s) can be calculated with Eq. (3).

$$\eta = C t \rho \cdot 10^{-3} \quad (3)$$

where C is the constant of the Ubbelodhe capillary viscometer (mm²·s^{−2}), t is the time taken by the liquid front to pass from the upper to lower marks (s); ρ is the density of protein solution (kg·m^{−3}).

2.2.3.4. ζ -potential. The ζ -potential of emulsion droplets was measured using a ZetaSizer Nano ZS (Malvern Instruments Ltd., UK). The samples were diluted 1000 times with Milli-Q water. Each measurement was performed 3 times at room temperature (20 °C). The refractive and absorption indices used for the oil droplets were set to 1.461 and 0.001 respectively. Milli-Q water was used as dispersant with refractive index 1.330 and dielectric constant 80.4. The cell type was DTS1070.

2.2.3.5. Stability of cream at dynamic conditions. Recombined dairy creams made with 4.0 wt% WPI or WPA were put in a water bath to warm up to 40 °C. Then the samples were subjected to stirring with a

Turrax (IKA T25, Germany) at 3000 rpm for 15 min. During the described experiment, the droplet size distribution of the samples was measured every 5 min. Tests were also conducted at different stirring speeds, i.e. 3000, 9000 and 10000 rpm.

2.2.4. Interfacial properties

2.2.4.1. Interfacial tension measurements. The interfacial tension of the purified milk fat-water interface was determined using a Tracker Automated Droplet Tensiometer (ADT) (Teclis, France). The purified anhydrous milk fat was poured into the cuvette of this system. A temperature control module was used to keep the temperature of the fat at 40 °C. A pendent drop of water or the protein samples was formed at the tip of a motored syringe (Trajan, Australia), submerged in the oil phase. The surface area of the droplet was 20 mm². The density of the droplet fluid and anhydrous milk fat at 40 °C were determined using a density meter (DMA 5000, Anton Paar, Germany), and the values were 0.9922 g/mL and 0.9041 g/mL, respectively. The time evolution of the interfacial tension was monitored for 1 h.

2.2.4.2. Large amplitude oscillatory dilatation (LAOD). After monitoring the interfacial tension for 1 h, sinusoidal oscillatory area deformations were applied to the droplet interface. The oscillation frequency was set as 0.005 Hz, and an amplitude sweep was performed in which the amplitude was set to 5, 10, 15, 20, 25 and 30%. For each amplitude, 5 cycles of oscillation were applied, followed by 300 s of recovery. The middle 3 cycles were used for constructing Lissajous Plots. Lissajous Plots were made using the method described by Sagis and Fischer (2014). According to this method, the surface pressure, (π), is plotted against the relative area deformation (γ), in a cyclic plot. The deformation and surface pressure were calculated using Eq. (4) and Eq. (5):

$$\gamma = \frac{A_t - A_0}{A_0} \quad (4)$$

$$\pi = \sigma_t - \sigma_0 \quad (5)$$

where A_t and σ_t are the interfacial area and interfacial tension at time t ; A_0 and σ_0 are the initial interfacial area and interfacial tension. The Lissajous Plots were analyzed in terms of the dilatational moduli at minimum and large extension (E_{dEM} and E_{dEL} , respectively), and the dilatational moduli at minimum and large compression (E_{dCM} and E_{dCL} , respectively), introduced earlier by van Kempen, Schols, van der Linden, and Sagis (2013) and based on a scheme introduced by Ewoldt, Hosoi, and McKinley (2008).

Once the amplitude sweep was completed, a new droplet was formed and the test was repeated at a higher frequency. The frequencies applied in this study were 0.005, 0.01, 0.02, 0.05 and 0.1 Hz.

2.2.5. Statistical analysis

In this study, all samples were prepared at least in duplicates, and all tests were conducted at least twice. For the samples with the same protein, statistical differences among concentrations were checked by ANOVA analysis, followed by Tukey's post hoc test, and significant differences were marked with different letters. For the samples with the same concentration, statistical differences between WPI and WPA were checked by T-test, and significant differences were marked with an asterisk (*). All analyses were conducted by IBM SPSS 25 (IBM SPSS Inc., Chicago, IL, USA). Significance was set as $P < 0.05$.

3. Results and discussion

3.1. Hydrophobicity and particle size distribution of the protein samples

As shown in Fig. 1, the slope of WPA was much higher than WPI, which meant that WPA was more hydrophobic than WPI. This is the

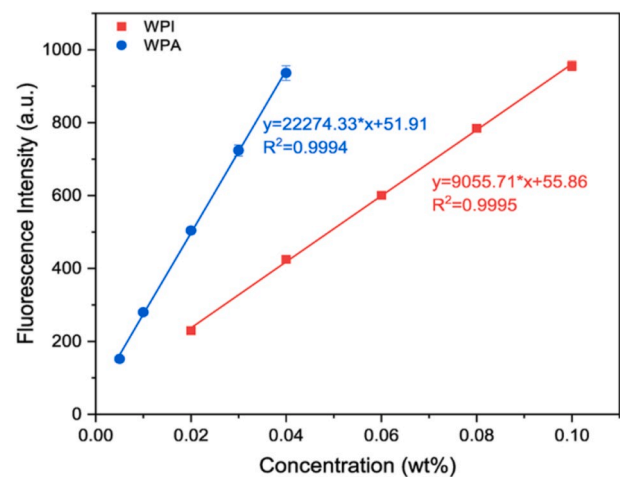


Fig. 1. Fluorescence intensity of WPI-ANS or WPA-ANS in PB buffer (0.01 M, pH 7.0, 20 °C) as a function of protein concentration, and their linear fits. The slope of the curve represents the relative hydrophobicity of the proteins.

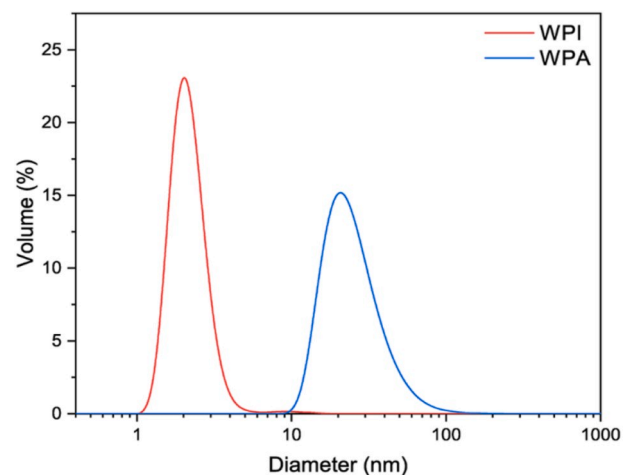


Fig. 2. Volume-weighted size distribution of WPI and WPA at room temperature (20 °C). Protein samples were diluted to 0.4 wt% with MilliQ water before testing.

result of the formation mechanism of WPA (Aguilera, 1995; Spiegel, 1999; Wijayanti, Bansal, & Deeth, 2014). First of all, during heat treatment globular proteins unfolded and reactive groups were exposed. The unfolded molecules unfolded, but the shear forces caused by stirring prevented the formation of a connected gel network, and individual protein aggregates were produced. As a result of the unfolding, more hydrophobic groups were exposed in WPA compared to native whey protein.

The scattering intensity scales with the size of particles to the power six, and the scattering of smaller particles can be somewhat obscured by the scattering of a few larger particles (Fig. S1). Therefore, the results are shown in terms of the volume-weight distribution rather than the intensity-weight distribution. As shown in Fig. 2, the size of WPI was smaller than 10 nm and distributed around 2 nm, while the peak of the size distribution of WPA was around 20 nm. These size distributions of WPI and WPA are in line with the results from Yang, Thielen, Berton-Carabin, van der Linden, and Sagis (2020).

3.2. Bulk stability

To explore whether aggregation of the protein can affect emulsion stability, the creaming rate of the droplets, their size distribution over

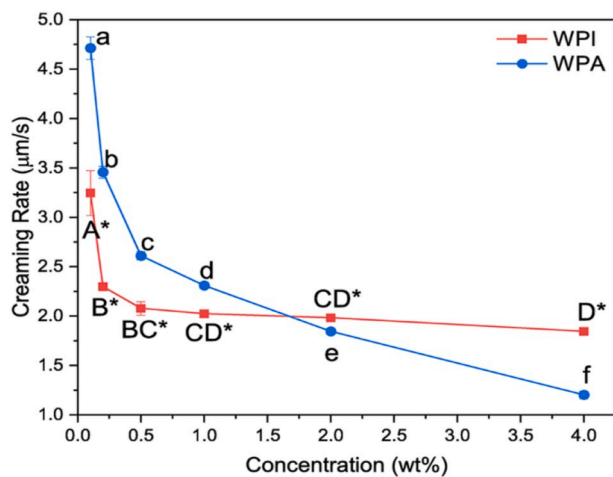


Fig. 3. Creaming rate of milk fat emulsions stabilized with WPI or WPA as a function of protein concentration, measured at room temperature (20 °C), and 10^3 g. An asterisk (*) is used to denote statistical differences between proteins at the same concentrations. Different letters mark the statistical differences between concentrations of the samples with the same protein.

time, their ζ -potential, and the effects of continuous phase viscosity on creaming were studied for milk fat emulsions stabilized by either WPI or WPA (at various concentrations). The emulsion tests were done with both purified and non-purified milk fat (results for the latter are included in the supplementary information; Fig. S2 - Fig. S5). There were only minor differences in stability between the two systems, which were mainly observed at low protein concentrations. At high protein concentrations, purified or non-purified milk fat emulsion systems had no difference in stability. Apparently, at low protein concentrations, the surface active components in anhydrous milk fat like mono- or diglycerides could compete with proteins for adsorption at the interface (Eric Dickinson, 1999; Dickinson & Tanai, 1992; Granger, Barey, Combe, Verschambre, & Cansell, 2003), and as a result, the stability was influenced by these surface active components. At a high protein concentration, the effects of proteins became more dominant in the system, and as a result, the differences between emulsions with purified milk fat or non-purified milk fat diminished. Therefore, the data of the interfacial characterization of the samples with purified milk fat and a high protein

concentration can also be used for interpreting stability data of the non-purified milk fat emulsions.

3.2.1. Creaming rate

The creaming rate of the emulsions decreased dramatically as the WPI or WPA concentration increased from 0.1 to 0.5 wt% (Fig. 3). Above 0.5 wt%, WPI-stabilized emulsions showed a nearly constant creaming rate. For WPA-stabilized emulsions, the creaming rate kept decreasing with increasing protein concentration. When the protein concentration was below 1.5 wt%, WPI-stabilized emulsions had a slower creaming rate than WPA-stabilized emulsions. Above 2.0 wt%, WPA-stabilized emulsions creamed more slowly than WPI-stabilized ones. To explain these observations, we investigated the droplet size distribution of the emulsions (section 3.2.2) and the continuous phase viscosity of the samples (section 3.2.3).

3.2.2. Droplet size distribution

The oil droplet size distribution of fresh milk fat emulsions (0d) and of samples centrifuged under conditions simulating a storage of 30 days (30d) was measured with (+SDS) and without SDS. This surfactant was added to unveil the presence of oil droplet aggregates, which would be disrupted by it. Our results suggest that at concentrations of 0.1 wt% and 0.2 wt%, neither WPI nor WPA could stabilize the emulsions. Fresh emulsions prepared with 0.1 wt% or 0.2 wt% WPI showed a single peak (Fig. 4). The 30d samples stabilized with these WPI concentrations had a bimodal distribution, while 30d + SDS showed a single peak, close to the original size distribution. Hence, for these emulsions the main destabilization mechanism was flocculation, and not coalescence. Similar behavior was observed for the samples with the same concentrations of WPA. However, during storage, coalescence or irreversible aggregation occurred in these emulsions, since the particle size distribution of the 30d WPA sample did not shift back to the original distribution after adding SDS. A significant shoulder remained at the right of the main peak.

At 0.5 wt%, both WPI and WPA could stabilize the emulsions against coalescence or aggregation, as the droplet size distribution of the 30d samples and 0d samples overlapped. Compared with WPI, the use of WPA always led to the formation of larger oil droplets, especially when the concentration was lower than 2.0 wt%. This is an indication that WPA had a weaker emulsifying ability. Above 2.0 wt%, the difference in mean oil droplet size $d_{4,3}$ (Table 1) between WPI- and WPA-stabilized

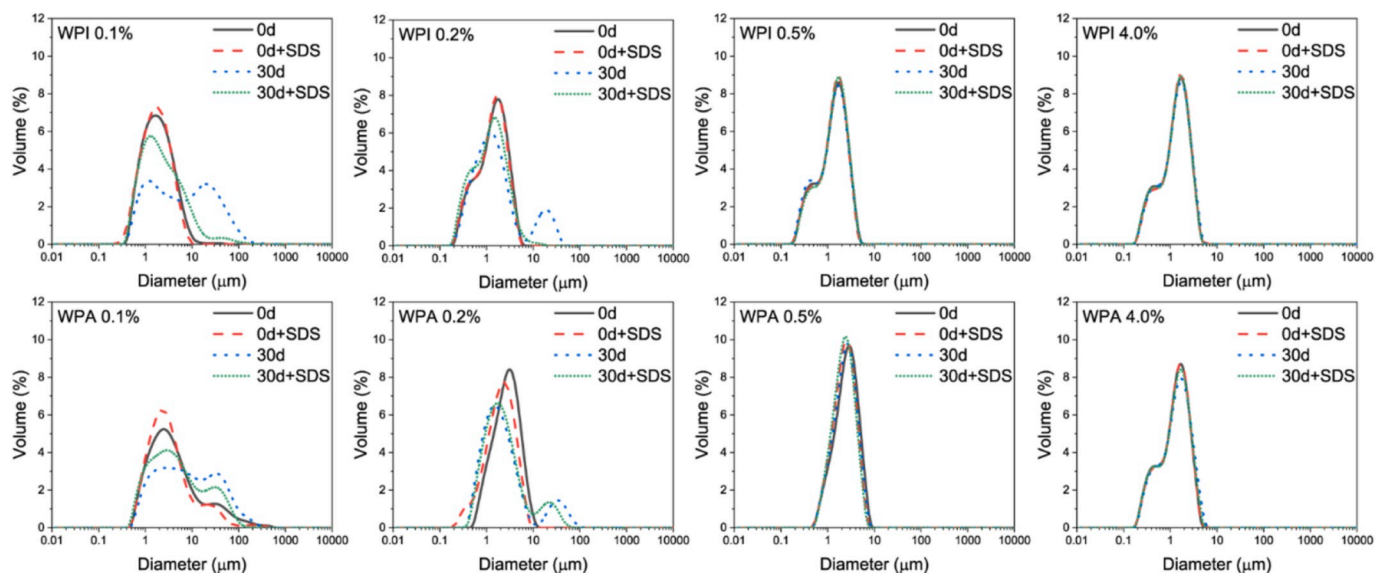


Fig. 4. Droplet size distribution of milk fat emulsions stabilized with WPI and WPA at various protein concentrations, including fresh samples (0d) with (+SDS) and without SDS, and samples after a simulated storage of 30 days (30d), with (+SDS) and without SDS.

Table 1

$d_{4,3}$ (μm) of fresh milk fat emulsions (0d) made from WPI or WPA. An asterisk (*) is used to indicate statistical differences between the two protein samples at the same concentrations. Different letters indicate statistical differences among different concentrations of the samples with the same protein.

Proteins	Concentrations (wt%)					
	0.1	0.2	0.5	1.0	2.0	4.0
WPI	2.31 \pm 0.29 ^{A*}	1.50 \pm 0.05 ^{B*}	1.42 \pm 0.07 ^{B*}	1.42 \pm 0.05 ^{B*}	1.39 \pm 0.04 ^{B*}	1.40 \pm 0.02 ^{B*}
WPA	13.00 \pm 3.76 ^a	2.92 \pm 0.10 ^b	2.63 \pm 0.01 ^b	1.92 \pm 0.05 ^b	1.49 \pm 0.01 ^b	1.37 \pm 0.01 ^b

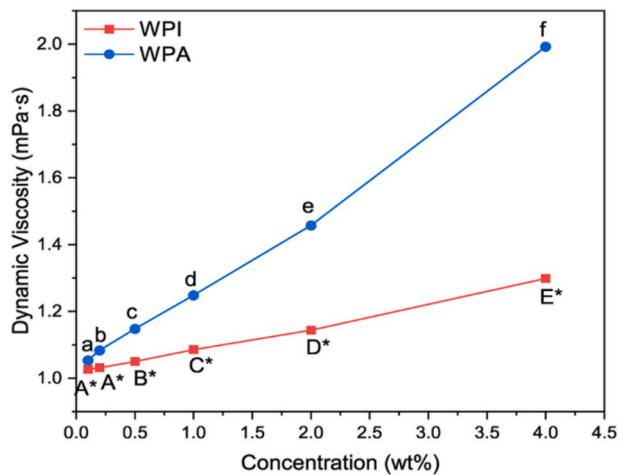


Fig. 5. Viscosity of WPI solutions and WPA dispersions at protein concentrations of 0.1 wt%, 0.2 wt%, 0.5 wt%, 1.0 wt%, 2.0 wt%, 4.0 wt%, at 20 °C. An asterisk (*) is used to indicate statistical differences between the two protein samples at the same concentrations. Different letters indicate statistical differences among different concentrations of the samples with the same protein.

emulsions was negligible ($<0.10 \mu\text{m}$).

3.2.3. Viscosity of the continuous phase

With an increase in protein concentration, the viscosity of both WPI and WPA solutions increased. Fig. 5 shows that the viscosity of the WPA solution was significantly higher than that of the WPI solution, particularly at the highest concentrations. This implied that WPA was a more efficient thickener. Combined with the data of the mean oil droplet size, $d_{4,3}$, shown in Table 1, the viscosity differences between WPI solutions and WPA dispersions could explain the differences in creaming rate we observed in section 3.2.1. At low concentrations (0.1 wt%–1.0 wt%), where the viscosities of the emulsions stabilized with the two proteins were still similar, the droplet size difference between the WPI- and WPA-stabilized emulsions was responsible for the higher creaming rate of the WPA emulsions. At higher concentrations (>1.0 wt%), the mean droplet sizes of the two emulsions were close, but the difference in viscosity of the continuous phase was much larger. As a result, the WPA emulsions had a lower creaming rate.

3.2.4. ζ -potential

The pH of the milk fat emulsions was approximately neutral (around 7), and at this pH whey protein is negatively charged. Upon increasing protein concentration from 0.1 to 4.0 wt%, the ζ -potential of WPI-stabilized emulsions gradually and significantly ($P < 0.05$) decreased from -39.13 ± 3.59 mV to -56.82 ± 1.68 mV (Fig. 6). In the same concentration range, the ζ -potential of WPA-stabilized emulsions decreased from -36.77 ± 1.06 mV to -60.23 ± 2.56 mV. Although at a concentration of 4.0 wt%, the values of the ζ -potential of the emulsions stabilized by the two proteins were significantly different, the difference

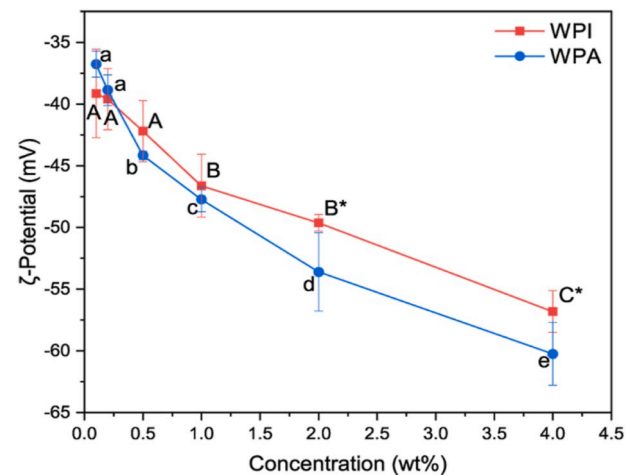


Fig. 6. ζ -potential of milk fat emulsions made with WPI or WPA at various protein concentrations, measured at 20 °C. The emulsions were 1000 times diluted by MilliQ water before testing. An asterisk (*) is used to indicate statistical differences between the two protein samples at the same concentrations. Different letters indicate statistical differences among different concentrations of the samples with the same protein.

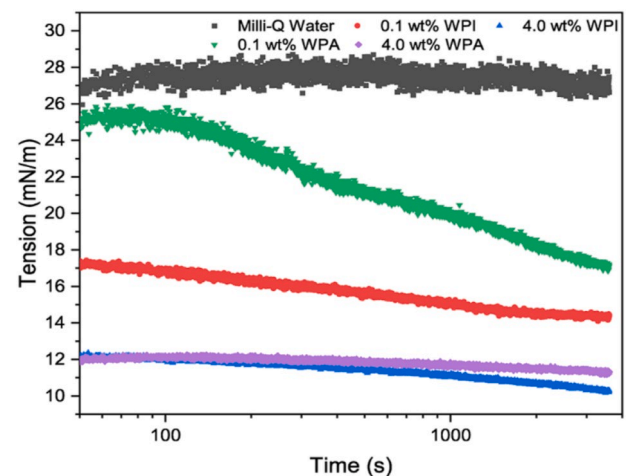


Fig. 7. Interfacial tension between anhydrous milk fat and a WPI solution or a WPA dispersion at 40 °C. Data before 50 s is not shown, since the droplet was not yet in thermal equilibrium with the continuous oil phase.

was marginal (<5 mV), and we can conclude that differences in stability between the two emulsions were unlikely to be related to differences in electrostatic repulsion among droplets.

3.3. Interfacial properties

All measurements of interfacial properties were done with purified milk fat, as the surface tension of the interface between non-purified milk fat and the protein solutions was too low, resulting in detachment of the droplet from the tip of the needle of the tensiometer. As a result of the much lower surface to volume ratio, tensiometry measurements are more sensitive to these impurities. As we pointed out above (section 3.2), the differences in macroscopic properties between purified and non-purified milk fat emulsions were negligible at higher protein concentrations, so interfacial data for purified milk fat can also be used for interpreting stability data of the non-purified milk fat emulsions.

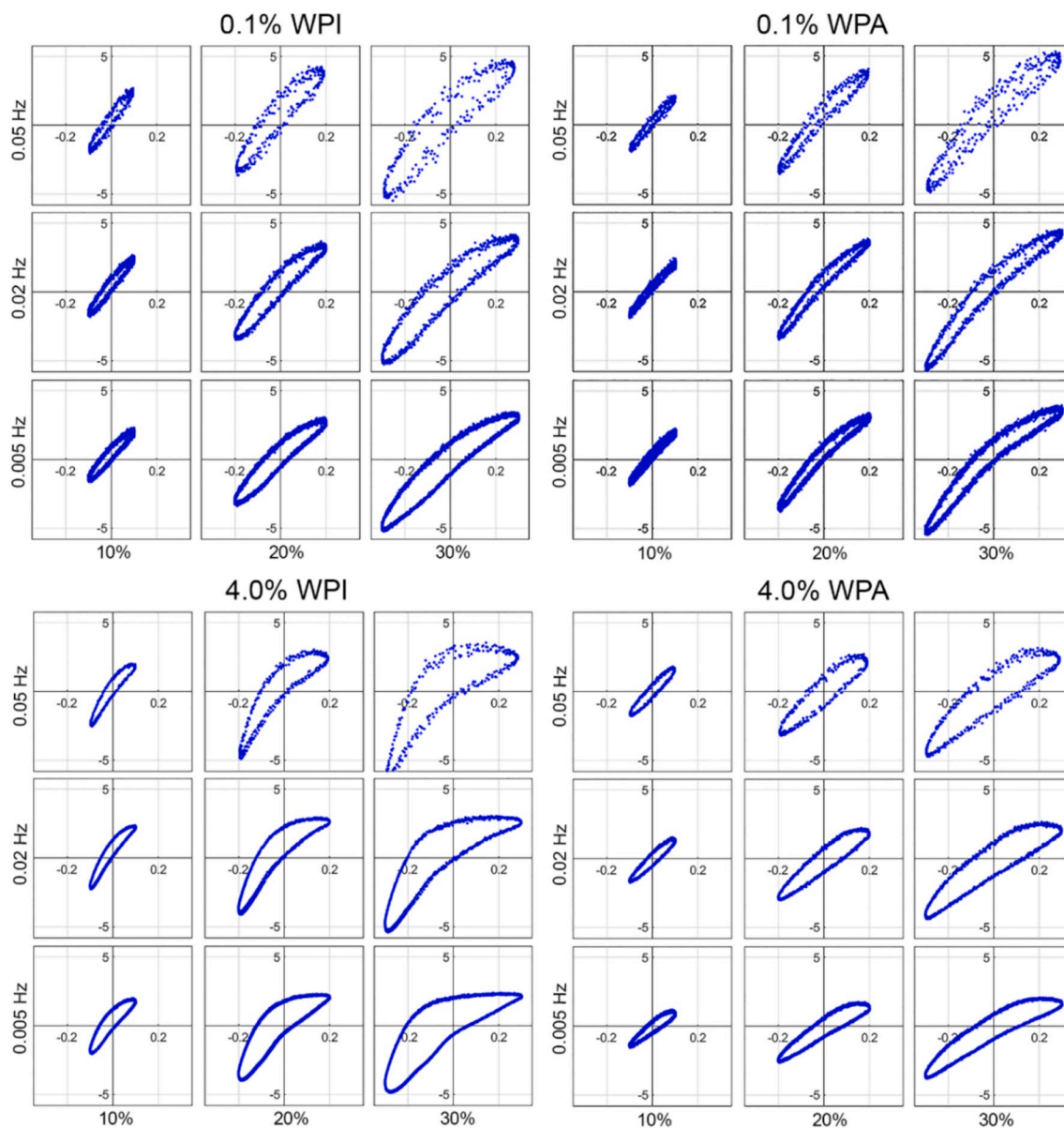


Fig. 8. Pipkin Plots showing the evolution of the surface pressure of WPI- and WPA-stabilized milk fat-water interfaces at protein concentrations of 0.1 and 4.0 wt%. Strain amplitude was varied from 10% to 30%. Frequency was varied from 0.005 Hz to 0.05 Hz.

3.3.1. Interfacial tension

The interfacial tension as a function of time, from 50 s to 3600 s, is shown in Fig. 7. The data obtained before 50 s is not shown, because the droplets had not yet reached their target temperature of 40 °C, and were therefore not in thermal equilibrium with the continuous oil phase. At a concentration of 0.1 wt%, WPI decreased the interfacial tension of the milk fat-water interface to a nearly constant value of about 15 mN/m in approximately 1000 s, while for WPA the interfacial tensions decreased much less and much more slowly, in spite of the fact that WPA has a higher surface hydrophobicity (see Fig. 1) and was therefore expected to more readily adsorb at the interface. It has been shown that molecular size can have a significant influence on the rate of adsorption (Beverung, Radke, & Blanch, 1999; Dybowska, 2011; Jung, Gunes, & Mezzenga, 2010; Sobhaninia, Nasirpour, Shahedi, & Golkar, 2017). Larger molecules and particles may diffuse towards and adsorb at the interface more slowly than smaller ones. Increased rigidity could also be a factor (Dybowska, 2011; Segall & Goff, 2002; Wijayanti et al., 2014), since it would cause aggregates to unfold more slowly and to a lesser extent at the interface. Beverung et al. (1999) showed that compared to smaller

size proteins, larger molecules need a higher surface coverage to achieve the same decrease in interfacial tension. All these would explain why at a low concentration, WPA reduced interfacial tension much slower than WPI. When the concentration increased to 4.0 wt%, the initial diffusion-controlled phase could not be observed, which meant at a high protein concentration, the interface quickly became saturated with WPI or WPA, within the 50 s start-up phase in which thermal equilibrium was not yet attained. Once the interfaces became saturated, WPI and WPA decreased the interfacial tension to a similar extent. This explains the nearly equal droplet size of the emulsions prepared at this protein concentration (Table 1 in section 3.2.2). During emulsion formation, convection contributed to the transfer of proteins to the interface, together with diffusion, quickly saturating the interfaces with proteins. Since WPI and WPA decreased interfacial tension to a similar extent at saturation, similar oil droplet sizes were obtained in emulsion formation when the energy input was the same.

3.3.2. Large amplitude oscillatory dilatation

In order to study the interfacial properties of WPI- and WPA-

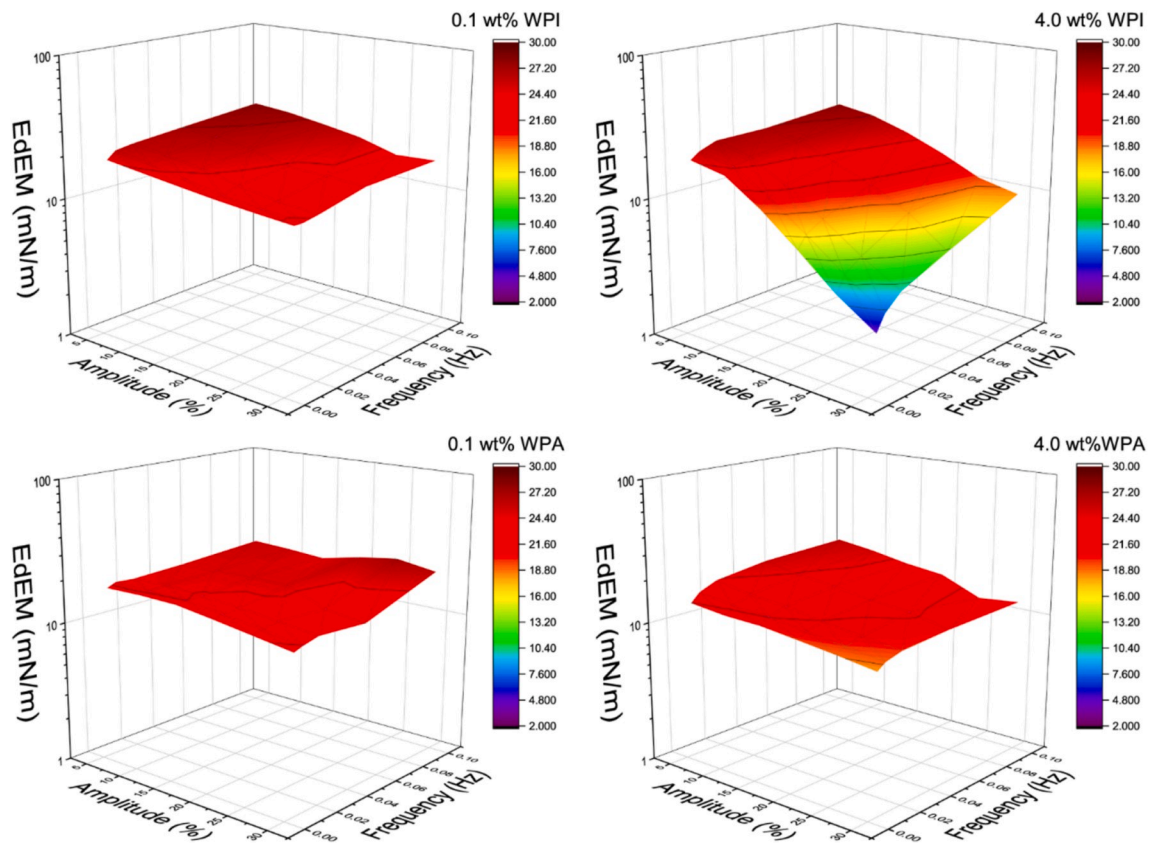


Fig. 9. E_{dEM} of WPI- and WPA-stabilized milk-fat-water interfaces at protein concentrations of 0.1 and 4.0 wt% as the function of strain amplitude and strain frequency.

stabilized milk fat-water interfaces in the nonlinear regime, large amplitude oscillatory dilatational (LAOD) measurements were performed, including amplitude and frequency sweeps. The obtained Pipkin plots are shown in Fig. 8. In general, the Lissajous plots became increasingly asymmetric with increasing amplitude, which meant that the response became progressively nonlinear. In expansion, strain softening was observed both for WPI- and WPA-stabilized interfaces, as evidenced from the decrease of the slope in the upper right quadrant of the plot. At a low concentration (0.1 wt%), the response of both WPI- and WPA-stabilized interfaces was dominated by the elastic contribution (Fig. 8), since the Lissajous plots were very narrow. The plots for the WPA-stabilized interfaces were narrower, indicating that the structure at the interface had a lower loss tangent and was relatively more solid-like. The stiffness of the two interfaces was however comparable at this concentration. At a high concentration (4.0 wt%), the strain softening behavior was more pronounced for WPI-stabilized interfaces. Particularly at 30% deformation, the plots for these interfaces had a high initial slope at the start of the expansion part of the cycle (the lower left corner of the plot), followed by an abrupt change in the slope, after which the slope was near zero. This pointed to intra-cycle yielding, which meant that the structure had been disrupted to such an extent that it started to flow, and the response became predominantly viscous. Compared with WPI, WPA-stabilized interfaces had a milder and more gradual strain softening in expansion, and a larger maximum linear strain. So, although the two types of interfaces appeared to be similar in stiffness, the WPA-stabilized interfaces were more stretchable and less brittle than the WPI-stabilized interfaces.

The Lissajous plots were analyzed using a method introduced by Ewoldt et al. (2008), and modified by van Kempen et al. (2013). The elastic modulus E_{dEM} (the tangent modulus in expansion at minimum strain) was calculated and plotted in Fig. 9. Additional plots for the modulus E_{dCM} (the tangent modulus in compression at minimum strain),

E_{dCL} (the secant modulus in compression at largest strain) and E_{dEL} (the secant modulus in expansion at largest strain) are provided in the supplementary information (Fig. S6 – Fig. S8). For E_{dEM} , there was no difference between WPI- and WPA-stabilized interfaces at a low concentration (Fig. 9). However, at a high concentration, the value of E_{dEM} of the WPI-stabilized interface decreased substantially as a function of amplitude, from a maximum value of 18.0 mN/m to a value of 2.6 mN/m (when the frequency was 0.005 Hz). This clearly showed the yielding of the structure, in which the interfacial behavior changed from viscoelastic solid to viscoelastic liquid behavior. The stronger frequency dependence observed for WPI at high strains could be attributed to an increased mobility within the interface, which led to shorter relaxation times, and as a result, the frequency range where the response was still frequency-dependent was shifted to higher frequencies. In contrast, the value of E_{dEM} of the WPA-stabilized interfaces showed a much smaller and more gradual decrease with increasing amplitude. This decrease was virtually independent of frequency, which implied that the interface retained a more viscoelastic solid like behavior, even at the highest amplitudes tested. At a high protein concentration, adsorption to and desorption from the interface could play a role in the response to oscillatory deformations. However, when plotting the elastic moduli as a function of frequency, in the linear regime, we observed a power law behavior ($E' \sim \omega^n$), with a value of the power n for WPI or WPA between 0.1 and 0.2 (Fig. S9). This was significantly lower than the value of $n = 0.5$, predicted by the Lucassen van den Tempel model, for interfaces in which the response is dominated by diffusion between bulk and interface (Lucassen & Van Den Tempel, 1972; Sagis et al., 2019). Combined with the low loss tangent (Table S1), the power law behavior we observed was indicative of an interface with a soft viscoelastic disordered solid structure, which implied that the response of WPI or WPA stabilized interfaces to dilatation was dominated by in-plane interactions.

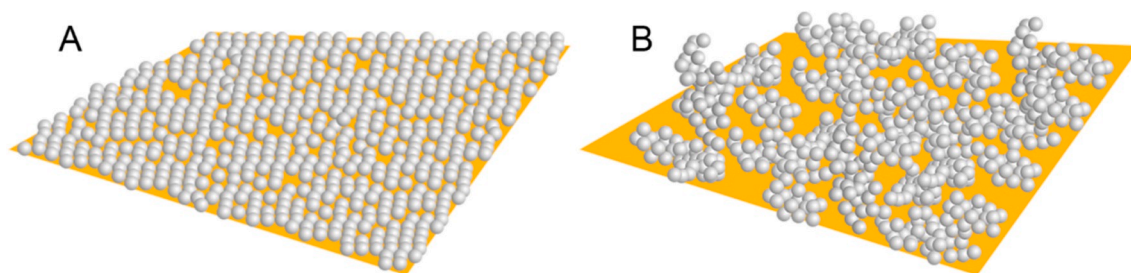


Fig. 10. Schematic representation of the (quasi-) 2d structure on oil-water interfaces formed by WPI (A), and the 3d structure formed by WPA (B).

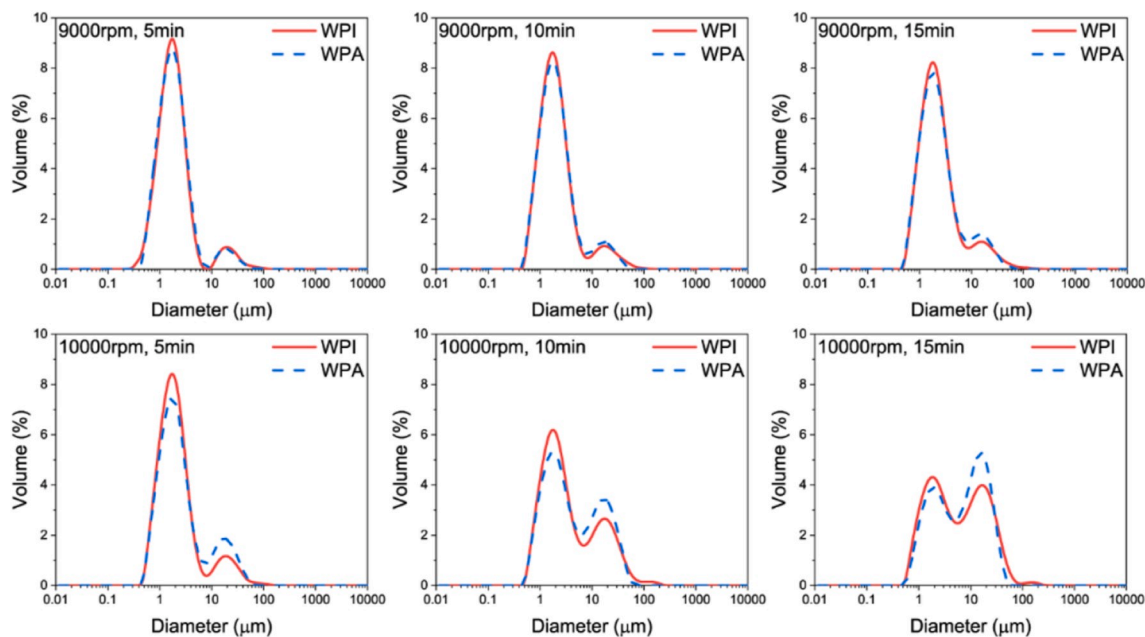


Fig. 11. Evolution of the droplet size distribution of emulsions made with 4.0 wt% WPI or WPA upon stirring at different speeds.

Based on the facts that, 1) WPA has a larger size than WPI; 2) at 4.0 wt%, WPI showed abrupt intra-cycle yielding followed by a predominantly viscous behavior at large expansion; and 3) the WPA interfacial layer had a larger maximum linear strain, and showed a more gradual softening in expansion and mild strain hardening in compression, we formulate the hypothesis that WPI might form a denser and more brittle (quasi-) 2d interfacial structure, and WPA might form a coarser and thicker 3d interfacial structure (Fig. 10). For WPI, molecules were further compressed and concentrated during the compression, which resulted in a higher connectivity among molecules. Subsequently, during the expansion, the interface initially showed a solid elastic response, followed by yielding, which was evidenced by a steep initial slope in the expansion part of Lissajous plots, followed by a near zero slope. For WPA, the coarser and thicker 3d structure led to a lower connectivity among aggregates. Consequently, the plots had a smaller initial slope and the interfaces retained more of their elastic behavior in the expansion part of the Lissajous plots, and only a gradual softening was found at the end of the expansion. These differences in structure between WPI and WPA-stabilized interfaces also implied that the WPI and WPA-stabilized emulsions may have differences at dynamic conditions. In the following, the stability of WPI and WPA-stabilized emulsions under dynamic conditions will be discussed in section 3.4.

3.4. Stability of the emulsions at dynamic conditions

The stability of the emulsions under dynamic conditions was tested by determining the droplet size distribution after stirring at various

speeds and stirring times (Fig. 11). Microscopic images of the samples after stirring are shown in the supplementary information (Fig. S10 and Fig. S11). At a stirring speed of 3000 rpm, both WPI- and WPA-stabilized emulsions were stable against coalescence (data is not shown). The emulsions started to destabilize once the stirring speed was increased to 9000 rpm, where another peak at around 20 μm could be observed in the size distribution. At 9000 rpm, the difference between WPI- and WPA-stabilized emulsions was still negligible. However, at 10000 rpm WPA, compared with WPI, always had a slightly higher peak at 11 μm and a lower peak at 1.5 μm. This meant that the WPA-stabilized emulsion was somewhat less resistant to the strong stirring than WPI, although the difference was quite small.

The results of section 3.3.2 proved that under dynamic conditions the interface stabilized by WPI displayed intra cycle yielding behavior and behaved more like a (quasi-) 2d viscoelastic liquid at large deformations, while the WPA layer did not show this yielding behavior, but a more gradual softening, retaining more of a solid-like behavior. As a result, the WPA interfacial layer could break at large and fast deformations, leading to exposure of parts of the interface, and to an increase in the rate of coalescence. Although at a high protein concentration, the interface formed by WPI was more brittle than the WPA interfacial layer, WPI could flow and remain at the interface during fast and large deformation. Consequently, it could protect oil droplets by a mechanism similar to the Marangoni effect demonstrated for small molecular surfactants.

4. Conclusion

In this study the stability of milk fat emulsions prepared with WPI or WPA is explained in terms of bulk and interfacial properties. For emulsions with a low protein content, WPI displays better emulsifying ability than WPA, even though in the linear regime the viscoelastic properties of interfaces formed by the two proteins are similar. At high concentrations, WPA can stabilize emulsions better than WPI, as WPA can thicken the continuous phase of the emulsion more effectively. However, emulsions made with WPA are less stable when they are subjected to vigorous stirring. This may be caused by the fact that the WPI-stabilized interface has a denser and more brittle (quasi-) 2d structure. At large deformations, the WPI-stabilized interface shows yielding, thus preventing coalescence by a mechanism similar to the Marangoni effect. On the other hand, WPA forms a coarser and thicker 3d interfacial structure that is more solid-like, but may break at a large deformation. The results presented here are not only useful for developing more stable recombined dairy products, but also shed light on the effect of heat-induced whey protein modification on emulsifying ability and emulsion stability.

Declaration of competing interest

The authors declare that no competing interest exist.

CRediT authorship contribution statement

Xilong Zhou: Investigation, Formal analysis, Validation, Data curation, Writing - original draft, Visualization. **Guido Sala:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration. **Leonard M.C. Sagis:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration.

Acknowledgement

We appreciate the kind donation of the raw materials from Friesland Campina (Wageningen, Netherlands) and the funding from China Scholarship Council (CSC NO. 201706350123). We would also like to thank Naomi Arita Merino and Remco Kornet for general discussions and suggestions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2020.106100>.

References

- Aguilera, J. M. (1995). Gelation of whey proteins: Chemical and rheological changes during phase transition in food. *Food Technology (Chicago)*, 49(10), 83–89.
- Beverung, C., Radke, C. J., & Blanch, H. W. (1999). Protein adsorption at the oil/water interface: Characterization of adsorption kinetics by dynamic interfacial tension measurements. *Biophysical Chemistry*, 81(1), 59–80.
- Chen, M., Sala, G., Meinders, M. B., van Valenberg, H. J., van der Linden, E., & Sagis, L. M. (2017). Interfacial properties, thin film stability and foam stability of casein micelle dispersions. *Colloids and Surfaces B: Biointerfaces*, 149, 56–63.
- Dalgleish, D. G. (2006). Food emulsions—their structures and structure-forming properties. *Food Hydrocolloids*, 20(4), 415–422.
- Damodaran, S. (2005). Protein stabilization of emulsions and foams. *Journal of Food Science*, 70(3), R54–R66.
- Davis, J., & Foegeding, E. (2004). Foaming and interfacial properties of polymerized whey protein isolate. *Journal of Food Science*, 69(5), C404–C410.
- Destribats, M., Rouvet, M., Gehin-Delval, C., Schmitt, C., & Binks, B. P. (2014). Emulsions stabilised by whey protein microgel particles: Towards food-grade pickering emulsions. *Soft Matter*, 10(36), 6941–6954.
- Dickinson, E. (1999). Adsorbed protein layers at fluid interfaces: Interactions, structure and surface rheology. *Colloids and Surfaces B: Biointerfaces*, 15(2), 161–176.
- Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloids and Surfaces B: Biointerfaces*, 20(3), 197–210.
- Dickinson, E., & Tanai, S. (1992). Protein displacement from the emulsion droplet surface by oil-soluble and water-soluble surfactants. *Journal of Agricultural and Food Chemistry*, 40(2), 179–183.
- Dybowska, B. E. (2011). Whey protein-stabilized emulsion properties in relation to thermal modification of the continuous phase. *Journal of Food Engineering*, 104(1), 81–88.
- Ewoldt, R. H., Hosoi, A., & McKinley, G. H. (2008). New measures for characterizing nonlinear viscoelasticity in large amplitude oscillatory shear. *Journal of Rheology*, 52(6), 1427–1458.
- Foley, J., & O'Connell, C. (1990). Comparative emulsifying properties of sodium caseinate and whey protein isolate in 18% oil in aqueous systems. *Journal of Dairy Research*, 57(3), 377–391.
- Fredrick, E., Heyman, B., Moens, K., Fischer, S., Verwijlen, T., Moldenaers, P., et al. (2013). Monoacylglycerols in dairy recombined cream: II. The effect on partial coalescence and whipping properties. *Food Research International*, 51(2), 936–945.
- Granger, C., Barey, P., Combe, N., Veschambre, P., & Cansell, M. (2003). Influence of the fat characteristics on the physicochemical behavior of oil-in-water emulsions based on milk proteins-glycerol esters mixtures. *Colloids and Surfaces B: Biointerfaces*, 32(4), 353–363.
- Han, J., Zhou, X., Cao, J., Wang, Y., Sun, B., Li, Y., et al. (2018). Microstructural evolution of whipped cream in whipping process observed by confocal laser scanning microscopy. *International Journal of Food Properties*, 21(1), 593–605.
- Hoffman, J. R., & Falvo, M. J. (2004). Protein—which is best? *Journal of Sports Science & Medicine*, 3(3), 118.
- Jiang, Y., Chen, J., Deng, C., Suuronen, E. J., & Zhong, Z. (2014). Click hydrogels, microgels and nanogels: Emerging platforms for drug delivery and tissue engineering. *Biomaterials*, 35(18), 4969–4985.
- Jung, J.-M., Gunes, D. Z., & Mezzenga, R. (2010). Interfacial activity and interfacial shear rheology of native β -lactoglobulin monomers and their heat-induced fibers. *Langmuir*, 26(19), 15366–15375.
- van Kempen, S. E., Schols, H. A., van der Linden, E., & Sagis, L. M. (2013). Non-linear surface dilatational rheology as a tool for understanding microstructures of air/water interfaces stabilized by oligofructose fatty acid esters. *Soft Matter*, 9(40), 9579–9592.
- Lam, R. S., & Nickerson, M. T. (2015). The effect of pH and temperature pre-treatments on the physicochemical and emulsifying properties of whey protein isolate. *LWT—Food Science and Technology*, 60(1), 427–434.
- van Lent, K., Le, C. T., Vanlerberghe, B., & Van der Meeren, P. (2008). Effect of formulation on the emulsion and whipping properties of recombined dairy cream. *International Dairy Journal*, 18(10–11), 1003–1010.
- Lucassen, J., & Van Den Tempel, M. (1972). Dynamic measurements of dilational properties of a liquid interface. *Chemical Engineering Science*, 27(6), 1283–1291.
- Millqvist-Fureby, A., Elofsson, U., & Bergenstahl, B. (2001). Surface composition of spray-dried milk protein-stabilised emulsions in relation to pre-heat treatment of proteins. *Colloids and Surfaces B: Biointerfaces*, 21(1–3), 47–58.
- Mortensen, B. (2016). *Anhydrous milk fat/butter oil and ghee*.
- Nicolai, T., Britten, M., & Schmitt, C. (2011). β -Lactoglobulin and WPI aggregates: Formation, structure and applications. *Food Hydrocolloids*, 25(8), 1945–1962.
- Nicolai, T., & Durand, D. (2013). Controlled food protein aggregation for new functionality. *Current Opinion in Colloid & Interface Science*, 18(4), 249–256.
- Petkov, J. T., Gurkov, T. D., Campbell, B. E., & Borwankar, R. P. (2000). Dilatational and shear elasticity of gel-like protein layers on air/water interface. *Langmuir*, 16(8), 3703–3711.
- Phan, T. T. Q., Moens, K., Le, T. T., Van der Meeren, P., & Dewettinck, K. (2014). Potential of milk fat globule membrane enriched materials to improve the whipping properties of recombined cream. *International Dairy Journal*, 39(1), 16–23.
- Rodríguez Patino, J. M., Rodríguez Niño, M. R., & Sánchez, C. C. (1999). Adsorption of whey protein isolate at the oil–water interface as a function of processing conditions: A rheokinetic study. *Journal of Agricultural and Food Chemistry*, 47(6), 2241–2248.
- Sagis, L. M., & Fischer, P. (2014). Nonlinear rheology of complex fluid–fluid interfaces. *Current Opinion in Colloid & Interface Science*, 19(6), 520–529.
- Sagis, L. M., Liu, B., Li, Y., Essers, J., Yang, J., Moghimiheirabadi, A., et al. (2019). Dynamic heterogeneity in complex interfaces of soft interface-dominated materials. *Scientific Reports*, 9(1), 2938.
- Sarkar, A., Murray, B., Holmes, M., Ettelaie, R., Abdalla, A., & Yang, X. (2016). In vitro digestion of pickering emulsions stabilized by soft whey protein microgel particles: Influence of thermal treatment. *Soft Matter*, 12(15), 3558–3569.
- Schmitt, C., Moitzi, C., Bovay, C., Rouvet, M., Bovetto, L., Donato, L., et al. (2010). Internal structure and colloidal behaviour of covalent whey protein microgels obtained by heat treatment. *Soft Matter*, 6(19), 4876.
- Schröder, A., Berton-Carabin, C., Venema, P., & Cornacchia, L. (2017). Interfacial properties of whey protein and whey protein hydrolysates and their influence on O/W emulsion stability. *Food Hydrocolloids*, 73, 129–140.
- Segall, K., & Goff, H. (2002). Secondary adsorption of milk proteins from the continuous phase to the oil–water interface in dairy emulsions. *International Dairy Journal*, 12(11), 889–897.
- Sobhaninia, M., Nasirpour, A., Shahedi, M., & Golkar, A. (2017). Oil-in-water emulsions stabilized by whey protein aggregates: Effect of aggregate size, pH of aggregation and emulsion pH. *Journal of Dispersion Science and Technology*, 38(9), 1366–1373.
- Spiegel, T. (1999). Whey protein aggregation under shear conditions—effects of lactose and heating temperature on aggregate size and structure. *International Journal of Food Science and Technology*, 34(5–6), 523–531.
- Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, 108, 303–318.

- Vanderghem, C., Danthine, S., Blecker, C., & Deroanne, C. (2007). Effect of proteose-peptone addition on some physico-chemical characteristics of recombined dairy creams. *International Dairy Journal*, 17(8), 889–895.
- Wan, Z., Yang, X., & Sagis, L. M. (2016). Nonlinear surface dilatational rheology and foaming behavior of protein and protein fibrillar aggregates in the presence of natural surfactant. *Langmuir*, 32(15), 3679–3690.
- Wijayanti, H. B., Bansal, N., & Deeth, H. C. (2014). Stability of whey proteins during thermal processing: A review. *Comprehensive Reviews in Food Science and Food Safety*, 13(6), 1235–1251.
- Wilde, P. (2000). Interfaces: Their role in foam and emulsion behaviour. *Current Opinion in Colloid & Interface Science*, 5(3–4), 176–181.
- Wooster, T. J., & Augustin, M. A. (2007). Rheology of whey protein–dextran conjugate films at the air/water interface. *Food Hydrocolloids*, 21(7), 1072–1080.
- Wu, J., Shi, M., Li, W., Zhao, L., Wang, Z., Yan, X., et al. (2015). Pickering emulsions stabilized by whey protein nanoparticles prepared by thermal cross-linking. *Colloids and Surfaces B: Biointerfaces*, 127, 96–104.
- Wu, S., Wang, G., Lu, Z., Li, Y., Zhou, X., Chen, L., et al. (2016). Effects of glycerol monostearate and Tween 80 on the physical properties and stability of recombined low-fat dairy cream. *Dairy Science & Technology*, 96(3), 377–390.
- Yang, J., Thielen, I., Berton-Carabin, C. C., van der Linden, E., & Sagis, L. M. (2020). Nonlinear interfacial rheology and atomic force microscopy of air-water interfaces stabilized by whey protein beads and their constituents. *Food Hydrocolloids*, 101, 105466.
- Yener, S., & van Valenberg, H. J. (2019). Characterisation of triacylglycerols from bovine milk fat fractions with MALDI-TOF-MS fragmentation. *Talanta*, 204, 533–541.
- Zhou, X., Chen, L., Han, J., Shi, M., Wang, Y., Zhang, L., et al. (2016). Stability and physical properties of recombined dairy cream: Effects of soybean lecithin. *International Journal of Food Properties*, 20(10), 2223–2233.