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Andrés L. Márquez & Jorge R. Wagner

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Rheology of Cream-like Emulsions Prepared with Soybean Milk and Low Trans Vegetable Fat

Andrés L. Márquez · Jorge R. Wagner

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Abstract The objective of this work was to study the effect of fat globule size and fat content on the rheological behavior of oil-in-water emulsions prepared with soybean milk, sunflower oil and low trans vegetable fat (LTF). Emulsions were prepared with 40 % w/w lipid phase (containing different proportions of LTF) and three different homogenization methods were used in order to obtain different globule sizes. Emulsions were subjected to controlled magnetic shaking until an increase in thickness. Partial coalescence was observed in all systems containing solid fat, which gave a rheopectic behavior and an increase in the viscoelastic parameters of the emulsions. Smaller particle sizes required higher shaking times to produce an increase in thickness, even though this did not lead to different final values of the viscoelastic parameters. The highest partial coalescence degree was observed at 50 % LTF in lipid phase, but the highest final viscoelastic parameters after shaking were observed at 100 % LTF in the lipid phase. The rheological behavior of these emulsions indicates that they could be a potential vegetable substitute for traditional dairy creams.

Keywords Creams · Rheology · Particle size · Partial coalescence · Vegetable fat

Introduction

Dairy creams are oil-in-water (o/w) emulsions with a dispersed lipid phase partially composed of solid fat. These emulsions have the characteristic property of increasing their thickness after whipping or shaking [1]. These rheological changes occur as a consequence of a partial coalescence process, by which fat globules form a rigid three-dimensional network [2–4]. This phenomenon requires the presence of a proportion of solid fat in the dispersed lipid phase, as fat globules are joined when a solid fat crystal from one globule penetrates into the liquid oil portion of another globule [5–7]. Thus, the degree of partial coalescence is highly dependant on the solid fat content [6, 8]. Other authors were able to imitate this behavior by developing model cream-like emulsions prepared with milk protein and vegetable fat [9].

The use of soybean milk for the formulation of cream-like emulsions with similar rheological behavior to dairy creams had been previously accomplished [10]. However, a high melting fraction of dairy fat mixed with sunflower oil was used as the dispersed lipid phase in order to imitate dairy creams; this is opposed to the objective of obtaining a healthy, cholesterol-free vegetable system. The alternative use of a vegetable fat in the development of a food system would also require a low content of trans fatty acids, as they increase the risk of heart diseases [11].

The objective of this work was to obtain o/w emulsions prepared with soybean milk, sunflower oil and low trans vegetable fat (LTF), in order to analyze the effect of fat globule size and LTF content in the lipid phase on the

A. L. Márquez (✉) · J. R. Wagner
Laboratorio de Investigación en Funcionalidad y Tecnología de Alimentos (LIFTA), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352 (B1876BXD) Bernal, Buenos Aires, Argentina
e-mail: almarquez@conicet.gov.ar

J. R. Wagner
e-mail: jwagner@unq.edu.ar

A. L. Márquez · J. R. Wagner
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

rheological behavior of these systems. In this way, partial coalescence process was studied in emulsions fully prepared with components that are not present in traditional dairy creams.

Experimental Procedures

Materials

Fluid soybean milk (provided by SOYANA SH; San Martín, Argentina), LTF (Delicrok LT1; provided by CALSA; Lanús, Argentina), refined sunflower oil (Molinos Río de la Plata S.A.; Avellaneda, Argentina), and xanthan gum (Parafarm; Buenos Aires, Argentina) were used to make emulsions.

Soybean milk contained 2.6 % w/w protein, 6.4 % w/w total solids and had a pH of 6.6. LTF was obtained by interesterification of natural fatty acids and fully hydrogenated fatty acids from vegetable oil (70 % cotton oil and 30 % soybean oil, approximately). The composition of LTF was: saturated fats, 50 % w/w; trans fats, 1 % w/w; monounsaturated fats, 13 % w/w; and polyunsaturated fats, 36 % w/w. The melting point of the LTF was 45 °C.

Preparation of Emulsions

All emulsions were prepared with soybean milk (containing 0.2 % w/w xanthan gum as stabilizer) as the continuous aqueous phase and 40 % w/w of dispersed lipid phase. Emulsions (sample weight, 100 g) with a lipid phase composed by different LTF contents (0, 25, 50, 75, and 100 % w/w) and the rest by sunflower oil, were homogenized using a rotor–stator homogenizer Ultra-turrax T-25 (IKA-Labor Technik; Staufen, Germany) with a S25-20NK-18G rotor (IKA-Labor Technik; Staufen, Germany) at 12,000 rpm for 1 min. Emulsions (sample weight, 100 g) containing a lipid phase with 100 % w/w LTF were also prepared with a rotor–stator homogenizer at 24,000 rpm for 1 min. A high pressure valve homogenizer Panda 2K (GEA Niro Soavi; Parma, Italy) at a pressure of 100 and 10 bar in the first and second valve, respectively, was also used to prepare emulsions with 100 % w/w LTF in lipid phase after a pre-homogenization of 300 g sample with a rotor–stator homogenizer at 12,000 rpm for 1 min was completed. LTF was previously melted and homogenizations were performed at 60 °C in order to avoid crystallization of the fat during the process. All preparations were done in triplicate. The emulsions were stored at 4 °C for 1 day before the corresponding characterizations.

Controlled Magnetic Shaking

Emulsions were shaken with a magnetic stirrer at a speed of 150 rpm and room temperature (21 ± 1 °C). Samples were shaken until the stirring rod stopped as a consequence of thickness increase in the system. All emulsions that were homogenized with a rotor–stator homogenizer at 12,000 rpm were shaken for 1.5 min independently of their thickness increase, because at higher times they were destabilized by phase inversion.

Optical Microscopy

Micrographs were obtained with an optical microscope operating at 400× magnification and using an adapted digital camera (Canon A570 IS; Malaysia) at 4× optical zoom.

Particle Size Distributions

Particle size distributions of the emulsions were obtained with a particle analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd.; Worcestershire, UK). The De Brouckere, volume-weighted, moment mean diameter (d_{43}) was obtained from the volume particle size distributions. Samples were diluted in water in the dispersion system (Hydro 2000MU) at a speed of 2,000 rpm. In order to determine the presence of flocs, additional measurements of emulsions diluted in buffer with 1 % SDS as the dissociating agent [12] were performed.

Solid Fat Content

Solid fat content of the emulsions was analyzed by nuclear magnetic resonance (NMR) using a pulsed NMR spectrometer (Minispec-mq20, Bruker; Germany). Solid fat content values were corrected by subtracting the result given by the emulsion without LTF in the lipid phase. Measurements were done at 21 °C in all cases.

Differential Scanning Calorimetry (DSC)

Thermograms of the emulsions were obtained using a MDSC Q-200 instrument (TA Instruments; New Castle, DE, USA). Samples were enclosed in hermetically sealed aluminium pans and then cooled until 4 °C at 10 °C/min (with an isotherm at that temperature for 10 min) and heated until 70 °C at 5 °C/min. Melting enthalpy of the systems was calculated above 21 °C from the thermograms obtained and used as a parallel parameter of solid fat content.

Rheology

Flow behavior and oscillatory rheology of the emulsions were studied using an AR-G2 rheometer (TA Instruments; New Castle, DE, USA) with a cone-and-plate geometry (gap, 55 μm ; cone diameter, 40 mm; cone angle, 2°). Temperature (21 °C) was controlled with a water bath (Julabo ACW100, Julabo Labortechnik; Seelbach, Germany) associated with the rheometer. Flow behavior was analyzed by increasing the shear rate from 0.1 to 10 s^{-1} over 210 s, then keeping it constant at 10 s^{-1} for 60 s, and finally decreasing it from 10 to 0.1 s^{-1} over 210 s.

Experimental data for oscillatory rheology of shaken and not shaken emulsions were obtained by recording the storage or elastic modulus (G') and the loss or viscous modulus (G'') at an oscillation frequency of 1 Hz, within the linear viscoelasticity range.

Statistical Analysis

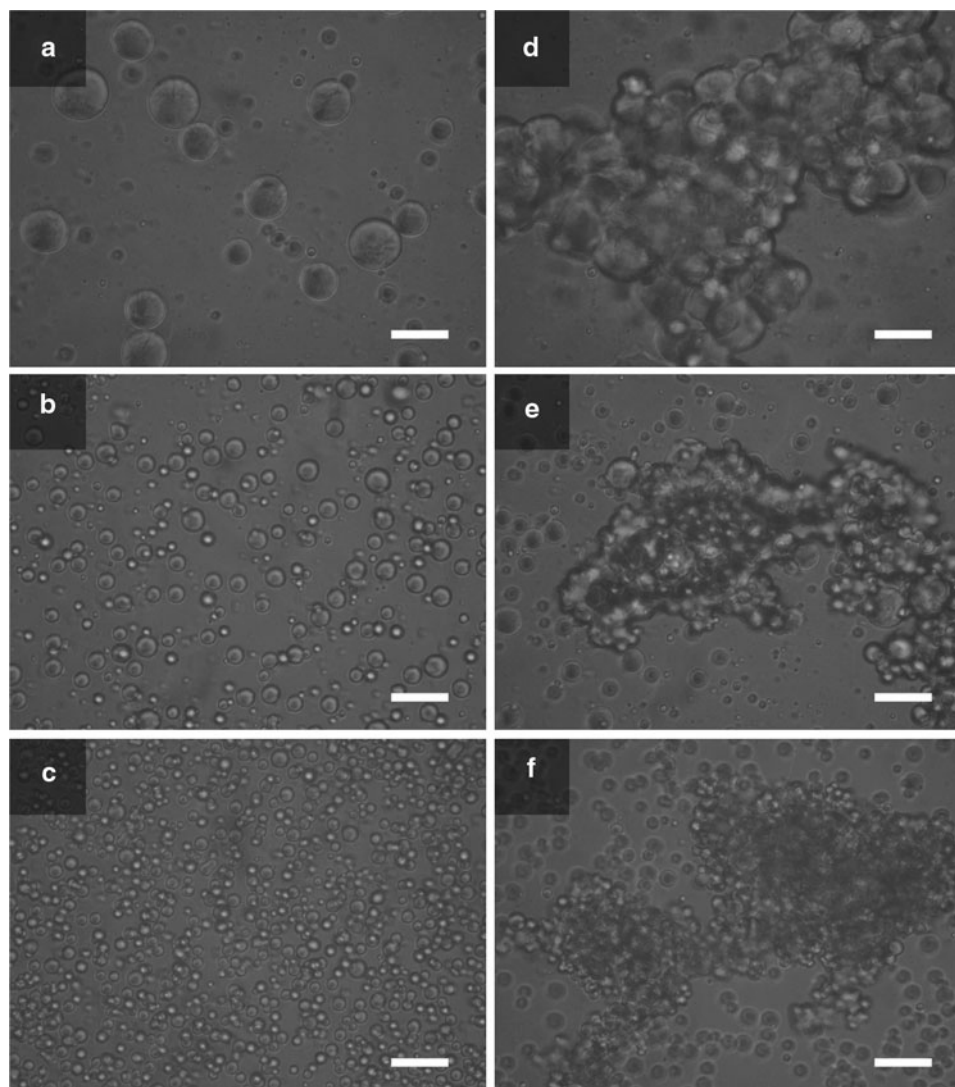
The statistical analysis was performed by analysis of variance and test of least significant difference ($P < 0.05$) using the statistical program Statgraphics Plus 5.1. Two or three independent replicates were measured at least two times in each experiment.

Results and Discussion

Effect of Globule Size

Figure 1 shows the optical micrographs corresponding to emulsions prepared with different homogenization methods. Smaller globule sizes were observed as a consequence of higher homogenization energy (Fig. 1a–c). These observations were confirmed by the analysis of particle size

Fig. 1 Optical micrographs of o/w emulsions with 100 % LTF in lipid phase (1:10 dilution): **a** homogenized with a rotor–stator homogenizer at 12,000 rpm; **b** homogenized with a rotor–stator homogenizer at 24,000 rpm; **c** homogenized with a valve homogenizer at 100 bar; **d** homogenized with a rotor–stator homogenizer at 12,000 rpm and shaken for 1.5 min; **e** homogenized with a rotor–stator homogenizer at 24,000 rpm and shaken for 9 min; **f** homogenized with a valve homogenizer at 100 bar and shaken for 33 min. Bar 20 μm



distributions (Fig. 2) and the corresponding d_{43} values (Table 1), comparing the results obtained for non-shaken samples. The emulsion prepared with a rotor–stator homogenizer at 12,000 rpm showed a multimodal distribution with three defined populations, with the higher mode being in the middle at 10.5 μm (Fig. 2a). With regard to the emulsion homogenized with a rotor–stator homogenizer at 24,000 rpm, it showed a bimodal distribution with the major population at 7 μm (Fig. 2b). On the other hand, the emulsion obtained with a valve homogenizer at 100 bar had one defined population at 4 μm with a shoulder corresponding to smaller particles (Fig. 2c). Moreover, determinations made with samples diluted in buffer with 1 % SDS, as a deflocculating agent, only showed a significant decrease in the d_{43} value in the system prepared with the valve homogenizer ($d_{43} = 3.46 \pm 0.02 \mu\text{m}$; $P < 0.05$), indicating the presence of flocs that are stable under the measuring conditions. This result can be explained by the smaller particle sizes produced by high pressure homogenization, which increases the concentration of globules and thus their interaction, favoring flocculation, and the shortage of surface-active components, which would not be enough to cover the created interface, leading to bridging flocculation [5].

Emulsions prepared with different homogenization methods required different shaking times to reach a consistency that was firm enough to stop the stirring rod. It was observed that the smaller the particle sizes the higher the required shaking time: 1.5 min for the rotor–stator homogenizer at 12,000 rpm; 9 min for the rotor–stator homogenizer at 24,000 rpm; and 33 min for the valve homogenizer at 100 bar. The increase in thickness was attributed to the formation of aggregates because of partial coalescence between globules, as it is commonly observed in dairy creams [2–4]. These aggregates were observed by optical microscopy (Fig. 1d–f) and this effect was manifested by the increase in d_{43} values after shaking (Table 1). Although the size of aggregates decreased with the size of individual globules in the system, the relative increase in the d_{43} value was higher at smaller initial particle sizes (e.g., the highest d_{43} value increased 297 % and the lowest d_{43} value increased 666 %), indicating a higher aggregation degree as a consequence of a higher interaction between globules. The emulsion prepared with a rotor–stator homogenizer at 12,000 rpm had a rapid increase in thickness. An increase in the proportion of larger particles at the expense of the two populations corresponding to smaller globule sizes was observed (Fig. 2a). With regard to the emulsion prepared with rotor–stator homogenizer at 24,000 rpm, the increase in thickness and aggregation was slower than in the system homogenized at 12,000 rpm (Fig. 2b). Finally, the emulsion obtained with valve homogenizer required a much higher shaking time than the

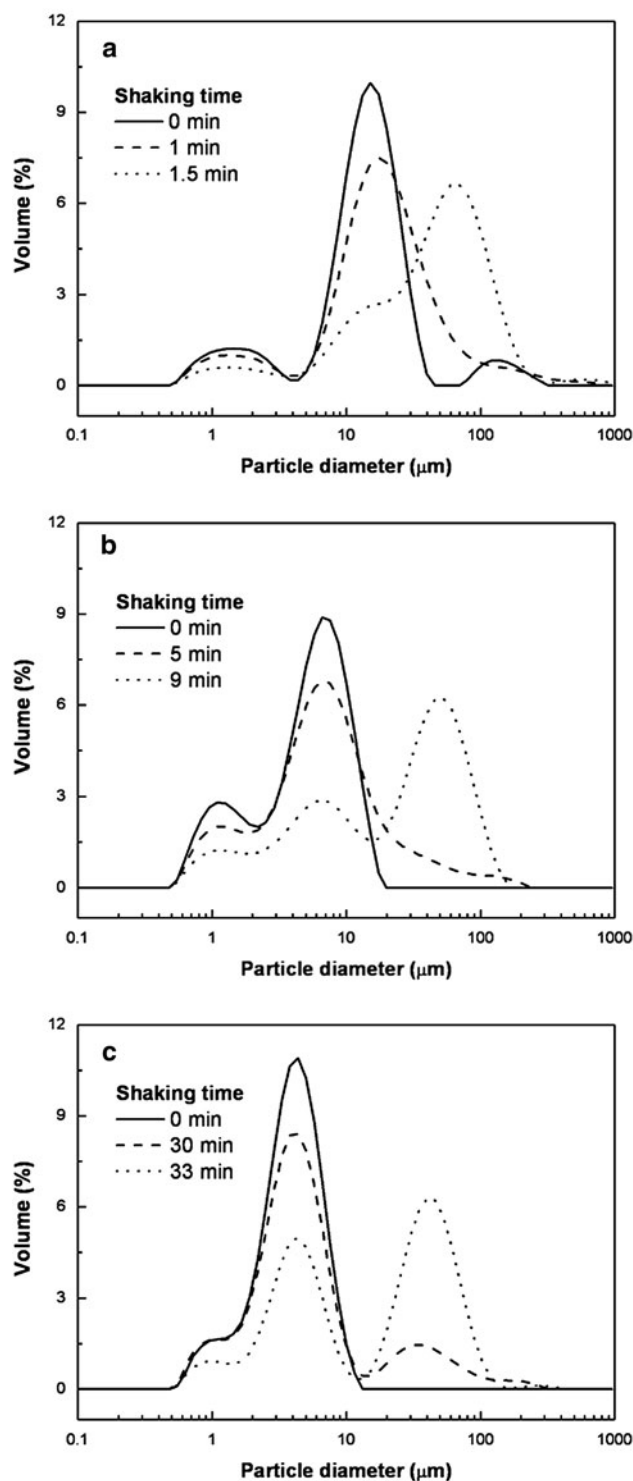


Fig. 2 Effect of shaking time on the particle size distribution of o/w emulsions with 100 % LTF in lipid phase: **a** homogenized with a rotor–stator homogenizer at 12,000 rpm; **b** homogenized with a rotor–stator homogenizer at 24,000 rpm; **c** homogenized with valve homogenizer at 100 bar

other samples to produce thickness increase, not observing aggregation until after 25 min (Fig. 2c). These results indicate that the higher the globule size, the higher the

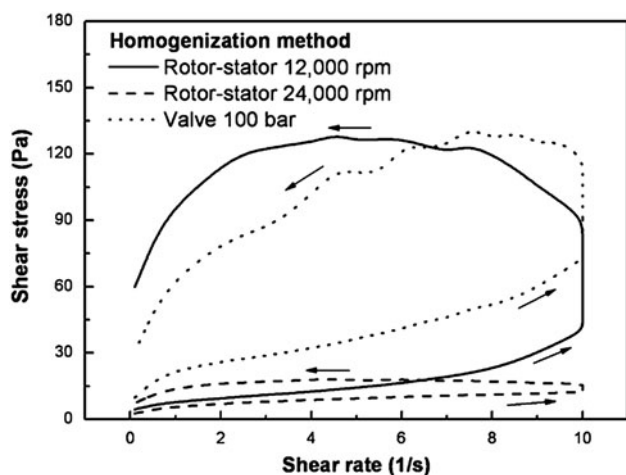
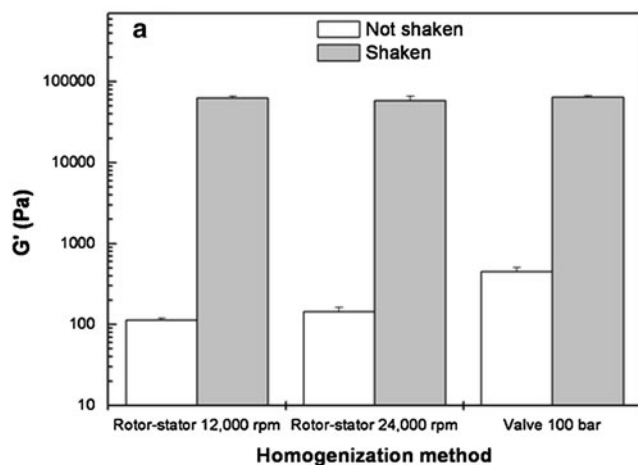
Table 1 Effect of the homogenization method and shaking on mean particle diameter (d_{43}) of o/w emulsions with 100 % LTF in lipid phase

Homogenization method	d_{43} (μm) ^{A,C}	
	Not shaken	Shaken ^B
Rotor–stator 12,000 rpm	20.10 \pm 0.74 a ■	59.79 \pm 2.27 a ●
Rotor–stator 24,000 rpm	5.42 \pm 0.07 b ■	31.10 \pm 0.58 b ●
Valve 100 bar	3.90 \pm 0.03 c ■	25.99 \pm 0.69 c ●

^A Values are means of three replicates \pm SD

^B Emulsions were shaken until the stirring rod stopped as a consequence of thickness increase

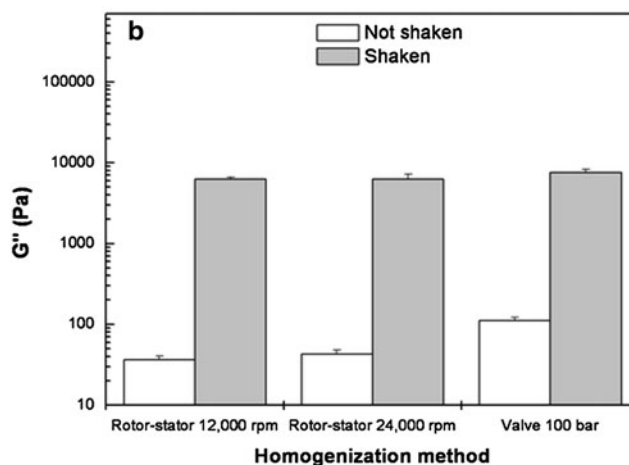
^C Mean values with different letters indicate that samples prepared with different homogenization methods are significantly different ($P < 0.05$). Mean values with different symbols indicate that a shaken sample is significantly different to its respective not shaken sample ($P < 0.05$)

**Fig. 3** Effect of the homogenization method on the flow behavior of o/w emulsions with 100 % LTF in lipid phase**Fig. 4** Effect of the homogenization method and shaking on the viscoelasticity of o/w emulsions with 100 % LTF in the lipid phase: **a** elastic modulus (G'); **b** viscous modulus (G''). Emulsions were

shaken until the stirring rod stopped as a consequence of thickness increase. Values are means of three replicates and error bars indicate SD

capture efficiency between globules to produce partial coalescence. Larger fat crystals can be formed in larger globules, leading to greater crystal protrusions through the interface and larger pore sizes in the crystal network. The greater protrusion distance increases the partial coalescence rate and the larger pore size increases the availability of liquid oil as a lubricating agent for the creation of a permanent junction [6]. Although no significant differences were detected in solid fat content and melting enthalpy for emulsions prepared with different homogenization methods ($P > 0.05$; data not shown), the different distribution of the solid fat as a consequence of the different globule sizes would explain the observed results.

Flow behavior of emulsions prepared with different homogenization methods can be observed in Fig. 3. In all cases, the way the shear stress increased with the increase in the shear rate indicates a pseudoplastic behavior. The rapid initial increase in shear stress could be attributed to a higher initial viscosity due to interactions between globules. At constant shear rate, these systems showed an increase in the shear stress, corresponding to a rheopectic behavior. The increase in viscosity observed at this stage was attributed to the partial coalescence process. A higher increase in shear stress in the systems was observed with the largest (rotor–stator homogenizer at 12,000 rpm) and the smallest (valve homogenizer at 100 bar) particles than in the emulsion with intermediate globule sizes (rotor–stator homogenizer at 24,000 rpm). The higher increase in shear stress in the system with largest particles can be explained by a higher capture efficiency between globules for the production of partial coalescence [6]; in this case, the increase in shear stress even continued when the shear rate started to be diminished. The emulsion with smallest particles showed a higher increase in shear stress than the other



shaken until the stirring rod stopped as a consequence of thickness increase. Values are means of three replicates and error bars indicate SD

Table 2 Effect of LTF content and shaking on mean particle diameter (d_{43}) of o/w emulsions homogenized with a rotor–stator homogenizer at 12,000 rpm

LTF content in lipid phase (% w/w)	d_{43} (μm) ^{A,C}	
	Not shaken	Shaken ^B
0	19.84 \pm 0.72 a ■	28.37 \pm 0.54 a ●
25	19.52 \pm 1.52 a ■	77.84 \pm 0.30 c ●
50	19.66 \pm 0.53 a ■	114.79 \pm 1.59 d ●
75	19.87 \pm 0.55 a ■	59.73 \pm 0.79 b ●
100	20.10 \pm 0.74 a ■	59.79 \pm 2.27 b ●

^A Values are means of three replicates \pm SD

^B Emulsions were shaken during 1.5 min

^C Mean values with different letters indicate that samples with different LTF content are significantly different ($P < 0.05$). Mean values with different symbols indicate that a shaken sample is significantly different to its respective not shaken sample ($P < 0.05$)

systems before constant shear rate; this result could be explained by the presence of flocs, as it was previously demonstrated for this emulsion, which would demand a higher initial stress due to the disruption of structures [5, 13].

Figure 4 shows the effect of the homogenization method and shaking on the viscoelasticity of the emulsions. In all cases higher G' than G'' values were observed, indicating higher elastic than viscous characteristics. The diminution of particle sizes led to higher G' and G'' values in non-shaken samples, due to the increase in interaction between globules as a consequence of their higher concentration [5]. Particularly, the emulsion prepared with valve homogenizer gave the highest G' and G'' values previously to shaking. This result may be related to the flocculation observed in this system. Flocculation increases the viscosity of an emulsion because the effective volume fraction

of a floc is greater than the sum of the volume fractions of the individual globules due to the presence of the continuous phase trapped within it [5]. Shaking produced an important increase in both viscoelastic parameters as a result of the partial coalescence process. The three studied emulsions showed similar G' and G'' values after shaking, but they required different shaking times to reach the same level of thickness. As it was previously explained, smaller globule sizes demanded higher shaking times due to a lower capture efficiency between globules. However, globule size did not seem to affect the final G' and G'' values, probably because they are mainly determined by the solid fat content, which was similar for the three systems.

Effect of LTF Content

The variation of LTF content in the lipid phase of the emulsions prepared with a rotor–stator homogenizer at 12,000 rpm did not affect the microstructure of the systems, as no significant differences were observed in the d_{43} values for non-shaken samples (Table 2) and the particle size distributions were all multimodal and similar to the initial distribution observed in Fig. 2a. However, LTF content did produce an effect on the size of aggregates after shaking during 1.5 min. In all cases, a significant increase in the d_{43} values after shaking was observed, but the magnitude of this increase depended on the LTF content (Table 2). The increase in the d_{43} value in the emulsion without LTF was attributed to ‘true’ coalescence rather than partial coalescence, because the presence of solid fat in the dispersed lipid phase is required for the latter phenomenon to occur [5–7]. Optical micrographs for this system also confirmed absence of partial coalescence, and flocculation was not detected by measurements made in

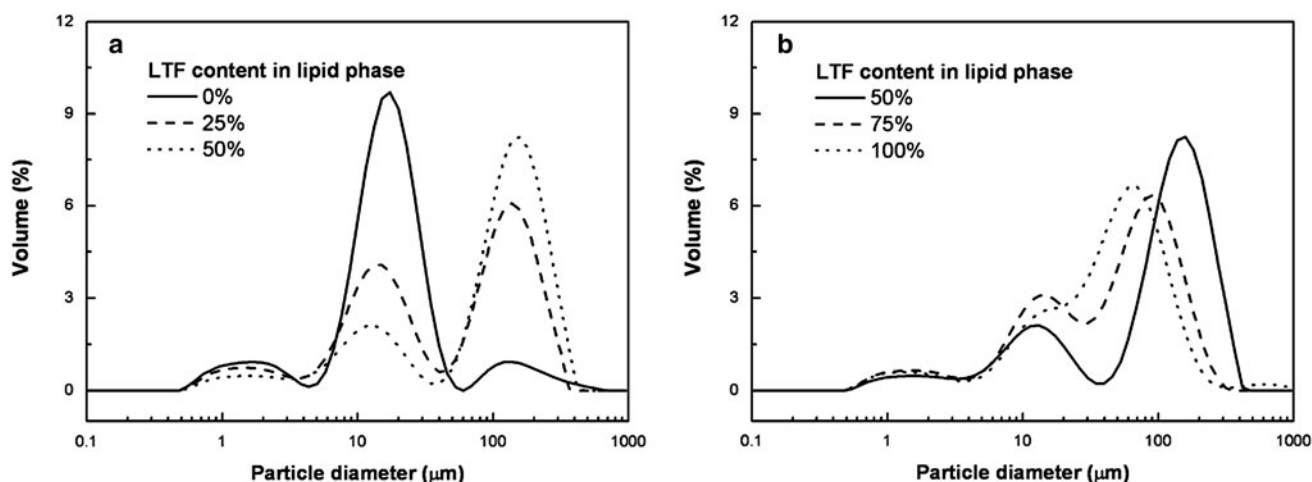


Fig. 5 Effect of LTF content on the particle size distribution of o/w emulsions homogenized with a rotor–stator homogenizer at 12,000 rpm and shaken for 1.5 min: **a** 0–50 % LTF in lipid phase; **b** 50–100 % LTF in lipid phase

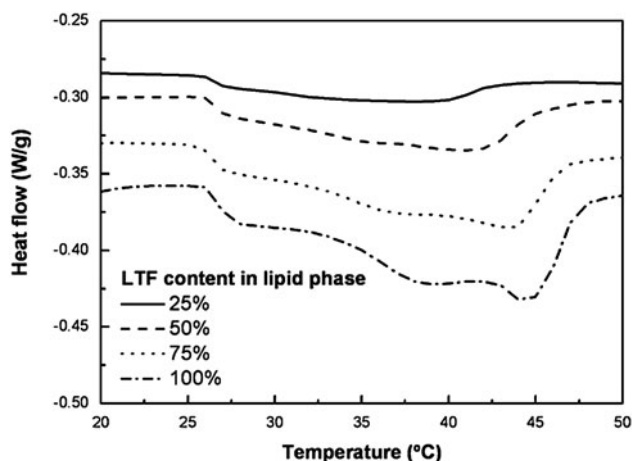
Table 3 Solid fat content and melting enthalpy of o/w emulsions homogenized with a rotor–stator homogenizer at 12,000 rpm

LTF content in lipid phase (% w/w)	Solid fat content in lipid phase (%) ^{A,C}	Melting enthalpy (J/g lipid phase) ^{B,C}
25	3.08 ± 0.13 a	5.45 ± 0.12 a
50	10.30 ± 0.49 b	12.89 ± 0.94 b
75	15.99 ± 0.20 c	19.78 ± 0.21 c
100	22.33 ± 0.99 d	28.15 ± 0.35 d

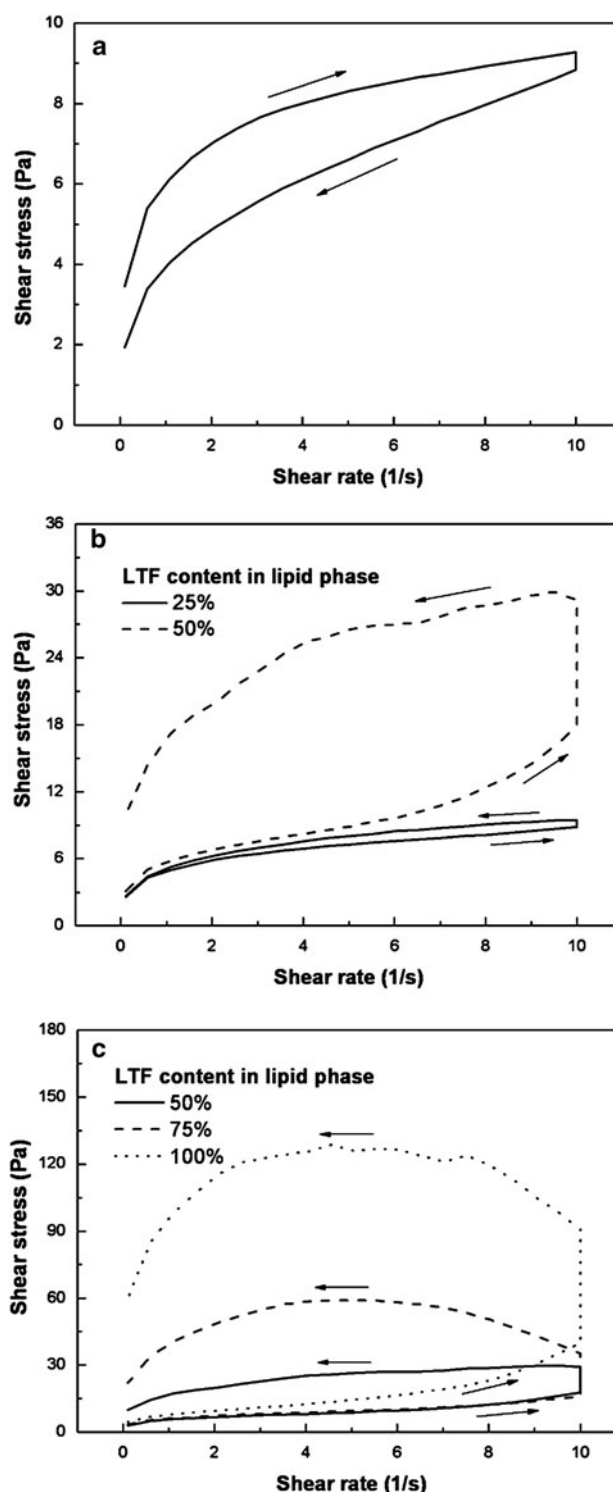
^A Solid fat content was determined by NMR at 21 °C

^B Melting enthalpy was determined by DSC above 21 °C

^C Values are means of two replicates ± SD. Mean values with different letters indicate that samples with different LTF content are significantly different ($P < 0.05$)

**Fig. 6** DSC thermograms of o/w emulsions with different LTF content, homogenized with a rotor–stator homogenizer at 12,000 rpm

presence of 1 % SDS after shaking (data not shown). The emulsion with the highest d_{43} value after shaking was the system with 50 % LTF in the lipid phase; higher or lower LTF contents led to the formation of smaller aggregates. This result can also be appreciated in the particle size distributions for shaken emulsions (Fig. 5). According to Fredrick et al. [6], both solid and liquid states in the dispersed lipid phase are necessary for the occurrence of partial coalescence, as it is produced when a solid fat crystal from one globule penetrates into the liquid oil portion of another globule. The increase in LTF content produced an increase in solid fat content at the shaking temperature, also confirmed by an increase in melting enthalpy (Table 3). The DSC thermograms also indicate a higher maximum melting point of the solid fat in the emulsions with higher LTF content (Fig. 6). The higher solid fat content would lead to higher number and size of fat crystals, producing greater crystal protrusions through the interface, but at the same time less and smaller pores in the crystal network. Thus, the diminution of partial coalescence degree below or above 50 % LTF in lipid phase

**Fig. 7** Flow behavior of o/w emulsions homogenized with a rotor–stator homogenizer at 12,000 rpm: **a** 0 % LTF in lipid phase; **b** 25–50 % LTF in lipid phase; **c** 50–100 % LTF in lipid phase

could be explained by the decrease in solid fat content at lower LTF percentages and the decrease in liquid oil quantity at higher LTF contents. Although, even at higher LTF percentages, the liquid oil quantity was higher than

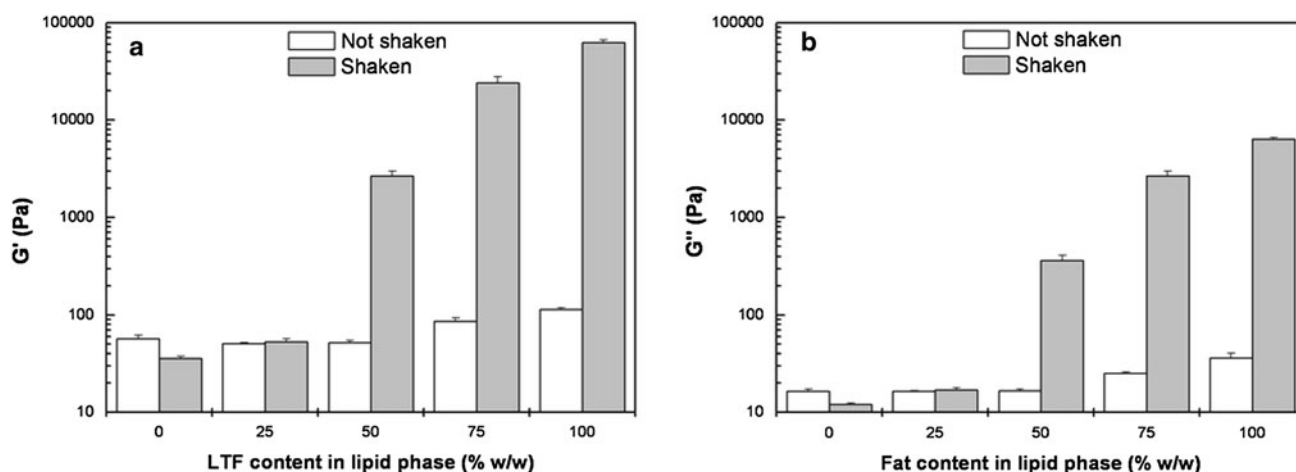


Fig. 8 Effect of LTF content and shaking on the viscoelasticity of o/w emulsions homogenized with a rotor–stator homogenizer at 12,000 rpm: **a** elastic modulus (G'); **b** viscous modulus (G'').

Emulsions were shaken for 1.5 min. Values are means of three replicates and *error bars* indicate SD

the solid fat content (Table 3), most of the solid fat would be located at the interface, as the interfacial free energy of the system is the lowest when the crystals are situated there [14]; this would reduce the space for liquid oil to interact with a solid fat crystal from another globule. It should also be mentioned that in all cases solid fat content did not vary after shaking (data not shown).

The effect of LTF content on the flow behavior of these emulsions is presented in Fig. 7. All these systems showed an initial pseudoplastic behavior. All emulsions showed a rheopectic behavior at constant shear rate, with the exception of the system without LTF, which showed a thixotropic behavior manifested by the decrease in shear stress (Fig. 7a). The lack of partial coalescence in this last emulsion due to the absence of solid fat plus the disruption of structures and diminution of number of droplets because of ‘true’ coalescence explains this result. The increase in LTF content produced a higher increase in shear stress (Fig. 7b–c) as a result of a higher solid fat content (Table 3). The emulsions with 50 and 75 % LTF in lipid phase showed similar increases in shear stress before constant shear rate, but the latter system reached higher shear stress values after that stage. Particularly, the emulsions with 75 and 100 % LTF in lipid phase showed an increase in shear stress even when the shear rate was decreased. Although it was previously observed that partial coalescence was favored at 50 % LTF in lipid phase (Table 2), the presence of a higher quantity of solid fat, also with higher maximum melting point (Fig. 6), may lead to the formation of a more rigid three-dimensional network, explaining the obtained results.

The viscoelasticity of the emulsions was also affected by the variation of LTF content, as it can be observed in Fig. 8. Before shaking, higher LTF contents led to higher

G' and G'' values (with $G' > G''$ in all cases), despite the fact that all these emulsions had similar particle sizes (Table 2). This result indicates that these emulsions contain enough dispersed lipid phase (40 %) to produce interaction between particles so that the viscosity of the system is affected by the composition and fat structure in the globules. In the case of the emulsion without LTF, shaking produced a decrease in G' and G'' values, attributed to the increase in the individual droplet sizes. This last result confirms that the increase in the d_{43} value after shaking in this system (Table 2) was attributed to ‘true’ coalescence and not to partial coalescence or flocculation, as the latter phenomena would have produced an increase in the viscosity [14]. For emulsions containing LTF, an increase in the viscoelastic parameters was observed after shaking during 1.5 min and higher final G' and G'' values were observed at higher LTF contents. These results are in agreement with the flow behavior data and indicate that LTF content was a main factor for viscoelasticity, probably because the partial coalescence produced in presence of higher solid fat content leads to the formation of structures with more solid-like characteristics.

Conclusions

The results obtained show that partial coalescence can be reproduced in emulsions prepared with soybean milk and a vegetable fat with low content of trans fatty acids. The use of different homogenization methods led to different fat globule sizes, which had an effect on the required shaking time to produce a thickness increase because of the variation of capture efficiency between globules. The increment of shear stress with shear rate was more pronounced at

highest globule sizes because of a higher capture efficiency, as well as at lowest globule sizes because of flocculation. The variation of solid fat content did not affect the globule sizes but gave different rheological behaviors. A higher partial coalescence degree did not always produce higher viscoelastic parameters after shaking, since the composition and fat structure in the globules were additional factors. These emulsions could be a potential vegetable substitute for traditional dairy creams due to their rheological behavior.

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