Research Article

Partial coalescence in double ($W_1/O/W_2$) emulsions prepared with skimmed milk, polyglycerol polyricinoleate, and different fats

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Partial coalescence was studied in double ($W_1/O/W_2$) emulsions prepared with skimmed milk, polyglycerol polyricinoleate (PGPR) as lipophilic emulsifier, and different fats. Microstructural and rheological analyses were performed. Encapsulation efficiency (as a parameter of inner water retention) and solid fat content were estimated by differential scanning calorimetry. The presence of PGPR in dispersed lipid phase promoted partial coalescence especially at higher concentration. This promotion of partial coalescence may have been produced by the increase of collision frequency due to protein displacement by PGPR at the outer interface and/or the increase of capture efficiency due to the modification of fat crystals by PGPR. Partial coalescence was also favored when inner water droplets were released as a consequence of an osmotic unbalance between inner and outer aqueous phases. Thus, the addition of glucose in dispersed aqueous phase decreased the partial coalescence degree due to the higher encapsulation efficiency given by the balanced osmotic pressures. With respect to the effect of the employed fat on partial coalescence, the obtained data indicate that the phenomenon was favored at higher solid fat content in lipid phase. Results led to the conclusion that inner osmolality, encapsulation efficiency, and inhibition of partial coalescence were correlated.

Practical applications: The originality of this work resides on boarding the subject of partial coalescence in $W_1/O/W_2$ emulsions. The studied systems are proposed as potential lipid-reduced substitutes of dairy creams, with the employment of alternative non-dairy fats such as low trans vegetable fat. The combined analysis of partial coalescence and encapsulation efficiency allowed studying their reciprocal effects and evaluating the potentiality of the systems for the encapsulation of hydrophilic compounds. The variations of relative osmotic pressures in inner and outer aqueous phases, lipophilic emulsifier concentration, and solid fat content may lead to desirable or undesirable rheological properties depending on the required texture of the food emulsion. The findings of this work could be an important step pointing to control the factors leading to partial coalescence in $W_1/O/W_2$ emulsions for food applications.

Keywords: Encapsulation efficiency / Fat / Partial coalescence / Polyglycerol polyricinoleate / $W_1/O/W_2$ emulsion

Received: October 28, 2016 / Revised: April 26, 2017 / Accepted: June 7, 2017

DOI: 10.1002/ejlt.201600447

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Abbreviations: BF, bovine fat; **DSC,** differential scanning calorimetry; **HS,** hydrogenated soybean oil; **LT,** low trans vegetable fat; **PGPR,** polyglycerol polyricinoleate; **SFC,** solid fat content; **SO,** sunflower oil

1 Introduction

Double or water-in-oil-in-water ($W_1/O/W_2$) emulsions are complex systems, composed of two aqueous phases and a lipid one. The first aqueous phase is dispersed in a lipid phase, which in turn is dispersed in a continuous second aqueous phase. $W_1/O/W_2$ emulsions are often prepared using the following two stages [1]: (i) preparation of a W_1/O

emulsion including a lipophilic emulsifier by a high energy homogenization method; (ii) dispersion of this W_1/O emulsion in a second aqueous phase (W_2) with a hydrophilic emulsifier, using lower homogenization energy. The use of different homogenization energies responds to the need of obtaining large enough oil droplets to contain smaller water droplets [2]. Because of the inclusion of water droplets within the dispersed phase, these systems have been proposed as a potential option for the elaboration of food emulsions with reduced lipid content [3] and/or encapsulation of hydrophilic compounds [4]. Recent works on $W_1/O/W_2$ emulsions pointed to increase the volume fraction of inner water droplets by osmotic swelling [5] and stabilize the same droplets by the inclusion of fat crystals in the lipid phase [6]. However, the use of solid fats for the preparation of $W_1/O/W_2$ emulsions and its effects on the properties of the system are still a matter of study.

Partial coalescence is a process by which two or more globules join together keeping part of their identity and eventually leading to the formation of a three-dimensional network [7–9]. This phenomenon requires the presence of solid particles within the globules, because aggregation occurs by a semi-solid junction [10, 11]. Partial coalescence is commonly observed in food emulsions like dairy cream, where part of the dispersed phase is composed by solid fat, altering the microstructure and rheology of the system. The process can be induced by the employment of mechanical work (e.g., whipping or shaking), increasing the collision frequency between globules [12–14]; or the application of a temperature cycle (cooling-heating-cooling) with partial melting of fats, increasing the capture efficiency because of the enrichment in fat crystals at the interface [14, 15]. Partial coalescence can also be favored in emulsions stabilized with proteins when there is competitive adsorption of other surfactants at the interface. The protein displacement can reduce the thickness and viscoelasticity of the interfacial film and change the repulsive interaction between globules, increasing capture efficiency and/or collision frequency [11]. Because formulation of $W_1/O/W_2$ emulsions requires the use of a lipophilic emulsifier for the preparation of the primary W_1/O emulsion, competitive adsorption is expected to occur in $W_1/O/W_2$ emulsions stabilized with proteins, and partial coalescence may be induced if the system includes solid fat. Furthermore, the lipophilic emulsifier may promote partial coalescence by modifying the properties and orientation of fat crystals and, thus, increasing capture efficiency [16].

The objective of this work was to analyze the partial coalescence process in $W_1/O/W_2$ emulsions prepared with skimmed milk, polyglycerol polyricinoleate (PGPR), and different fats. PGPR was selected as lipophilic emulsifier because it is widely used for the elaboration of W/O emulsions [17]. The microstructure and rheology of the systems were studied, analyzing the effects of glucose concentration in dispersed aqueous phase, PGPR concentration in lipid phase, and the employment of different fats. In this way, partial coalescence was studied in a food emulsion stabilized with dairy proteins and being reduced in lipid content due to the partial replacement of fat by inner water droplets.

2 Materials and methods

2.1 Materials

The following materials were used: skimmed cow milk powder (Mastellone Hnos. S.A.; Gral. Rodríguez, Argentina); distilled water; xanthan gum (Parafarm; Buenos Aires, Argentina); low trans vegetable fat (LT); refined bovine fat (BF); partially hydrogenated soybean oil (HS); refined sunflower oil (SO) (Molino Cañuelas SACIFIA; Cañuelas, Argentina); PGPR 90 (Grindsted-Danisco); glucose (Anedra; San Fernando, Argentina). LT, BF, HS, and PGPR were provided by CALSA (Lanús, Argentina). Glucose was of analytical grade.

The composition of the milk powder, according to the product label, was proteins, 52% w/w; carbohydrates, 35% w/w; lipids, 1% w/w. The humidity of milk powder was 5.93 ± 0.16% w/w. The melting points, origin information, and fatty acid composition of LT, BF, and HS have been published in a previous work [14].

2.2 Preparation of emulsions

$W_1/O/W_2$ emulsions were prepared by the two-stage emulsification method. The first stage consisted on the formulation of a W_1/O emulsion by homogenization of 20 g of aqueous phase (distilled water without or with glucose) and 80 g of lipid phase (fat with PGPR as lipophilic emulsifier) using a rotor-stator homogenizer Ultra-Turrax T-25 (IKA-Labortechnik; Staufen, Germany) with a S25-20NK-18G rotor (IKA-Labortechnik) at 24 000 rpm for 2 min. In the second stage, 20 g of primary W_1/O emulsion were mixed with 80 g of reconstituted cow milk (W_2, containing 10.0% w/w skimmed milk powder and 0.2% w/w xanthan gum as stabilizer) and the mixture was homogenized by Ultra-Turrax with a S25-20NK-18G rotor at 16 000 rpm for 1 min. Previously, the lipid phase was melted and the aqueous phases were heated (in all cases at 80°C) to avoid crystallization of the fat during both homogenization stages. Comparative O/W_2 emulsions were prepared containing 20 g of lipid phase (LT without or with 1.0% w/w PGPR) and 80 g of reconstituted cow milk at the conditions of the second homogenization stage. Immediately after their preparation, all samples were cooled in cold water and then stored at 7°C for 1 day before the corresponding characterizations. Emulsions were prepared in duplicate.

A base $W_1/O/W_2$ emulsion was prepared containing LT with 1.0% w/w PGPR in lipid phase and 4.9% w/w glucose

(corresponding to isotonic concentration with milk) in dispersed aqueous phase. The following parameters were varied keeping the other parameters equal to the base $W_1/O/W_2$ emulsion: glucose concentration in dispersed aqueous phase (no glucose, 4.9 and 9.8% w/w); PGPR concentration in lipid phase (0.5, 1.0, and 2.0% w/w); and the employed fat (LT, BF, HS, and SO).

2.3 Particle size distribution

The particle size distribution of the emulsions was determined by laser diffraction using 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 distribution; outer and inner d_{43} values were measured on $W_1/O/W_2$ and primary W_1/O emulsions, respectively. The refractive indices applied were 1.47 for oil phase and 1.33 for water phase. Samples were diluted in the dispersion unit (Hydro 2000MU, Malvern Instruments Ltd.) at a speed of 2000 rpm. O/W_2 and $W_1/O/W_2$ emulsions were measured after storage at 7°C for 1 day and their dilution was performed in water. W_1/O emulsions were measured immediately after homogenization and before lipid phase crystallization; and their dilution was performed in sunflower oil as described in a previous work [18]. All measurements were performed at least in duplicate.

2.4 Oscillatory rheology

The oscillatory rheology of the emulsions stored at 7°C for 1 day was studied using an AR-G2 rheometer (TA Instruments; New Castle, DE, USA) with a plate-and-plate geometry (gap, 1000 μm). Temperature (21°C) was controlled with a water bath (Julabo ACW100, Julabo Labortechnik; Seelbach, Germany) associated with the rheometer. Experimental data were obtained by recording the storage or elastic modulus (G') and the loss or viscous modulus (G") as a function of oscillation frequency (0.1–10 Hz) within the linear viscoelasticity range (0.2% strain). The complex modulus (G^*) and tan δ were calculated as $[(G')^2 + (G")^2]^{1/2}$ and G"/G', respectively. Tests were conducted at least in duplicate.

2.5 Optical microscopy

The microstructure of the emulsions stored at 7°C for 1 day was observed with an optical microscope operating at 400× magnification. Micrographs were obtained using an adapted digital camera (Canon A570 IS; Malaysia) at 4× optical zoom. A hot stage (40°C) was used to melt fat crystals and determine the occurrence of partial coalescence in emulsions.

2.6 Interfacial tension

The equilibrium interfacial tension given by reconstituted cow milk and lipid phase was measured with an automatic LAUDA TD 3 tensiometer (Lauda-Königshofen, Germany) using the du Noüy ring method. A total of 20 mL of milk were placed in a beaker (diameter, 6.0 cm) and the ring was lowered until its complete submersion in the aqueous phase. Then 30 mL of melted LT (without or with 1.0% w/w PGPR) were carefully added above the aqueous phase and the ring was automatically located at the position corresponding to the generated interface. A density difference between the phases ($\Delta\rho$) of 0.10 g/cm^3 was employed. Interfacial tension data were recorded until reaching equilibrium and the values corresponding to that condition were informed. Measurements were performed at 50°C, in order to avoid crystallization of the lipid phase. Tests were conducted in duplicate.

2.7 Interfacial rheology

The rheological properties of the interface between reconstituted cow milk and lipid phase were analyzed using an AR-G2 rheometer with a du Noüy ring geometry. A total of 30 mL of milk were placed in a beaker (diameter, 6.7 cm) and the ring was lowered until its complete submersion in the aqueous phase. Then 30 mL of melted LT (without or with 1.0% w/w PGPR) were carefully added above the aqueous phase and the ring was located at the position corresponding to the generated interface (gap, 10 900 μm). Interfacial rheology data were collected as a function of time (0–15 min) at constant frequency (0.1 Hz) and strain (5%). Mean G^* values were calculated using data from the time they reached a plateau to the end of the experiment (10–15 min). Measurements were performed at 50°C. Tests were conducted in duplicate.

2.8 Encapsulation efficiency

The encapsulation efficiency, defined as the percentage of dispersed aqueous phase of the primary W_1/O emulsion retained in the final $W_1/O/W_2$ emulsion [19], was estimated by differential scanning calorimetry (DSC) using a MDSC Q-200 instrument (TA Instruments). W_1/O and $W_1/O/W_2$ emulsions were enclosed in hermetically sealed aluminum pans immediately after their preparation and stored at 7°C for 1 day; then they were cooled until −85°C at 2.5°C/min. The encapsulation efficiency calculation by DSC has been previously proposed by other authors and can be also used to determine the relative size of W_1 droplets due to a variation of their freezing temperature [20]. According to Potier et al. [21], due to the small size of W_1 droplets, freezing of dispersed aqueous phase occurred with high undercooling, giving an exothermic transition at temperatures lower than −38°C. An additional exothermic transition appeared at a

higher temperature (about −20°C), corresponding to the freezing of the continuous aqueous phase in $W_1/O/W_2$ emulsions. Only the exothermic peaks corresponding to freezing of W_1 droplets were taken into account for encapsulation efficiency calculation. From those peaks, the freezing enthalpies in the $W_1/O/W_2$ emulsion (ΔH_d, expressed as J/g dispersed aqueous phase in primary W_1/O emulsion mixed with W_2) and its corresponding W_1/O emulsion (ΔH_s, expressed as J/g dispersed aqueous phase) were measured. Then the encapsulation efficiency was calculated as $(\Delta H_d/\Delta H_s) \times 100$. Measurements were performed in duplicate.

2.9 Solid fat content (SFC)

The SFC of the emulsions was estimated using a MDSC Q-200 instrument. Samples were enclosed in hermetically sealed aluminum pans immediately after their preparation and stored at 7°C for 1 day; then they were cooled until 1°C at 10°C/min (with an isotherm at that temperature for 10 min) and heated until 70°C at 5°C/min. SFC was calculated by a corrected method for SFC estimation by DSC [22]. The method was adapted as done in a previous work [14]. Measurements were performed in duplicate.

2.10 Statistical analysis

Analysis of variance and test of least significant difference ($p < 0.05$) were performed using the statistical program Statgraphics Plus 5.1 (Statistical Graphics Corp.; USA).

3 Results and discussion

3.1 Comparison of O/W_2 and $W_1/O/W_2$ emulsions

Two O/W_2 emulsions composed by LT, without and with 1.0% PGPR in lipid phase, and a $W_1/O/W_2$ emulsion prepared with the same fat and 1.0% PGPR in lipid phase were compared by the analysis of their microstructure and rheology (Fig. 1). The presence of PGPR produced significant changes in the particle size distribution of the O/W_2 emulsion. The absence of PGPR led to a d_{43} of 9.87 ± 0.16 μm with a bimodal distribution with a main population at 10 μm and a minor population at 1 μm. The addition of PGPR produced a d_{43} of 40.65 ± 2.62 μm with a trimodal distribution with an additional population at 50 μm (Fig. 1a) attributed to aggregation of fat globules by partial coalescence. The occurrence of this phenomenon was confirmed by optical microscopy showing the partially coalesced globules in the emulsion with PGPR (Fig. 2a) which were fused during gradual fat melting at 40°C (Fig. 2b and c), in contrast to the control emulsion without PGPR (Fig. 2d–f). The equilibrium interfacial tension given by milk and melted LT showed a significant decrease (from 3.00 ± 0.30 to 0.76 ± 0.12 mN/m) when 1.0% PGPR was included in the lipid phase, indicating the adsorption of the lipophilic emulsifier at the interface. Consequently, interfacial dairy proteins could be displaced by PGPR, as other authors have suggested [23]. However, according to interfacial rheology results, weakening of the interfacial film by the presence of PGPR was not detected, since the corresponding viscoelasticity parameter did not show significant differences (G^* values of $\sim 7 \times 10^{-6}$ Pa). Thus, partial coalescence may have been facilitated by the reduction of steric and electrostatic repulsions due to protein displacement at the interface without substantial weakening of the interfacial film, increasing the collision frequency between globules [11].

It should be considered that the addition of PGPR in the lipid phase of the O/W_2 emulsion did not produce a significant change in SFC at 7°C (data not shown), excluding it as a factor that could have led to partial coalescence during cold storage. Nevertheless, it has been suggested that lipophilic emulsifiers can modify the properties of fat crystals (growth, morphology, and/or polarity), changing their

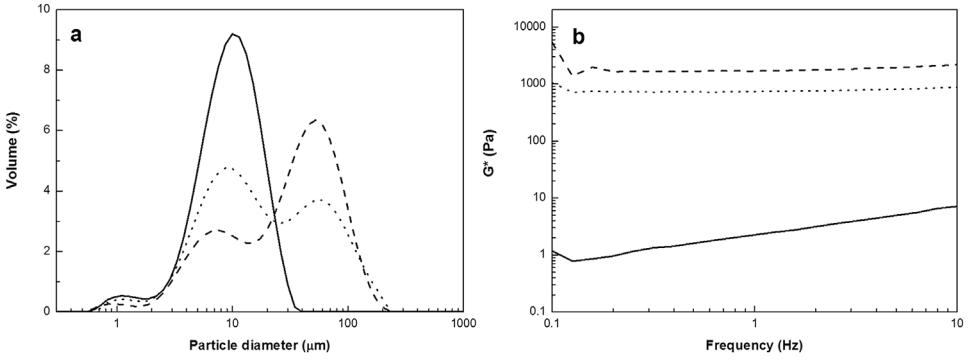

Figure 1. Comparison of O/W_2 emulsions prepared with LT without (——) and with (– – –) 1.0% PGPR in lipid phase and a $W_1/O/W_2$ emulsion prepared with LT, 1.0% PGPR in lipid phase and without glucose in dispersed aqueous phase (· · · ·). (a) Particle size distribution. (b) Oscillatory rheology.

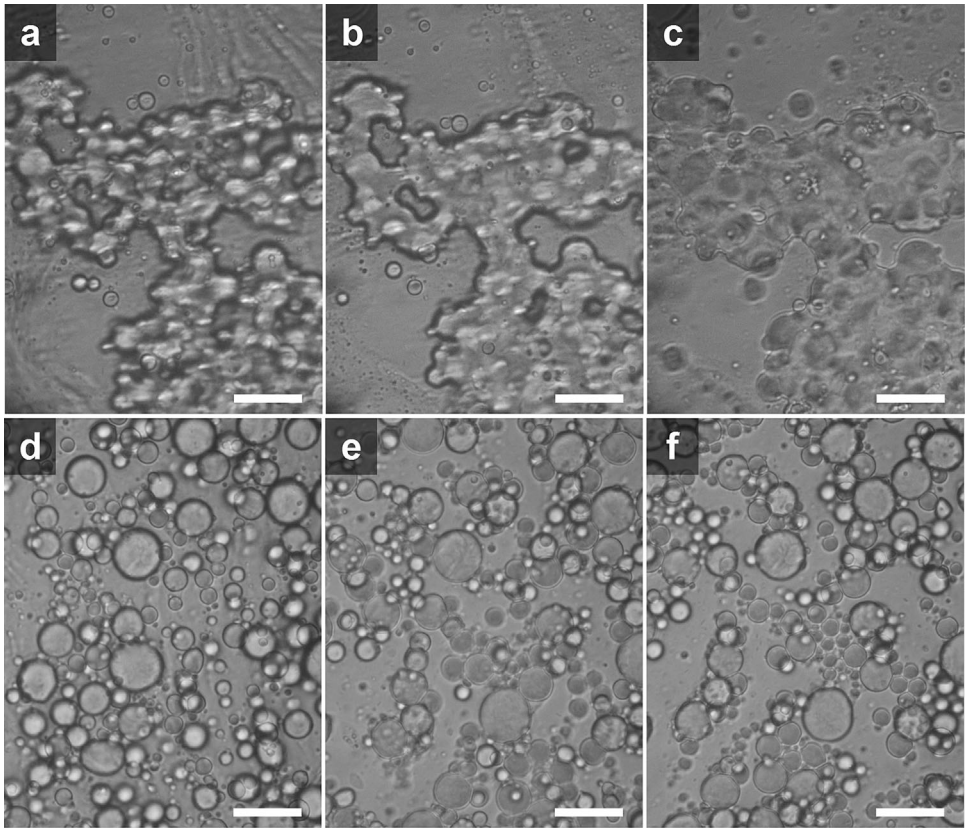

Figure 2. Optical micrographs of O/W_2 emulsions prepared with LT. System with 1.0% PGPR in lipid phase: (a) initial; (b) 2 min at 40°C; (c) 5 min at 40°C. System without PGPR in lipid phase: (d) initial; (e) 2 min at 40°C; (f) 5 min at 40°C. Bar = 20 μm.

orientation and promoting partial coalescence [16]. For instance, it has been reported that PGPR affects the polymorphism in tristearin during fat crystallization [24]. In this case, if fat crystals are modified in a way that partial coalescence is induced, the effect of PGPR would be the increase of capture efficiency when two globules collide.

With regard to the $W_1/O/W_2$ emulsion, its particle size distribution was trimodal but with a smaller population at 50 μm than the O/W_2 emulsion with PGPR (Fig. 1a). This result indicates that the presence of PGPR in the $W_1/O/W_2$ emulsion produced partial coalescence in a lower extent than that observed in the O/W_2 emulsion. These microstructural differences could be explained by a lower volume fraction of dispersed phase in the $W_1/O/W_2$ emulsion due to the release of W_1 droplets (as demonstrated below), leading to reduced partial coalescence degree because of the lower interaction between particles [25].

The rheological analysis of these emulsions, using G^* as viscoelasticity parameter, indicates that the O/W_2 emulsion with PGPR in lipid phase presented the highest G^* values at the whole frequency range (Fig. 1b). This result was attributed to the occurrence of partial coalescence when PGPR was added in the lipid phase. This phenomenon is known to increase the viscosity of emulsions because of the formation of a space-filling network restricting the molecular mobility of the system [11]. The $W_1/O/W_2$ emulsion gave slightly lower G^* values than those corresponding to the O/W_2 emulsion with PGPR (Fig. 1b), confirming its lower partial coalescence degree.

3.2 Effect of glucose concentration in $W_1/O/W_2$ emulsions

The effect of glucose concentration in dispersed aqueous phase on the particle size distribution of $W_1/O/W_2$ emulsions can be appreciated in Fig. 3a. In all cases a trimodal distribution was observed, but the emulsion with no glucose presented a bigger population of particles with diameters larger than 40 μm, so that its outer d_{43} value was higher than those obtained for the systems with glucose in dispersed aqueous phase (Table 1). In addition, the exothermic peaks obtained by DSC give information corresponding to the distribution of dispersed aqueous phase in W_1/O and $W_1/O/W_2$ emulsions (Fig. 3b). The decrease of water freezing temperatures with increasing glucose concentration in W_1/O emulsions would be given by the decrease of W_1 droplets size (inner d_{43}; Table 1) and the higher cryoprotective effect produced by the higher solute concentration [20]. A higher

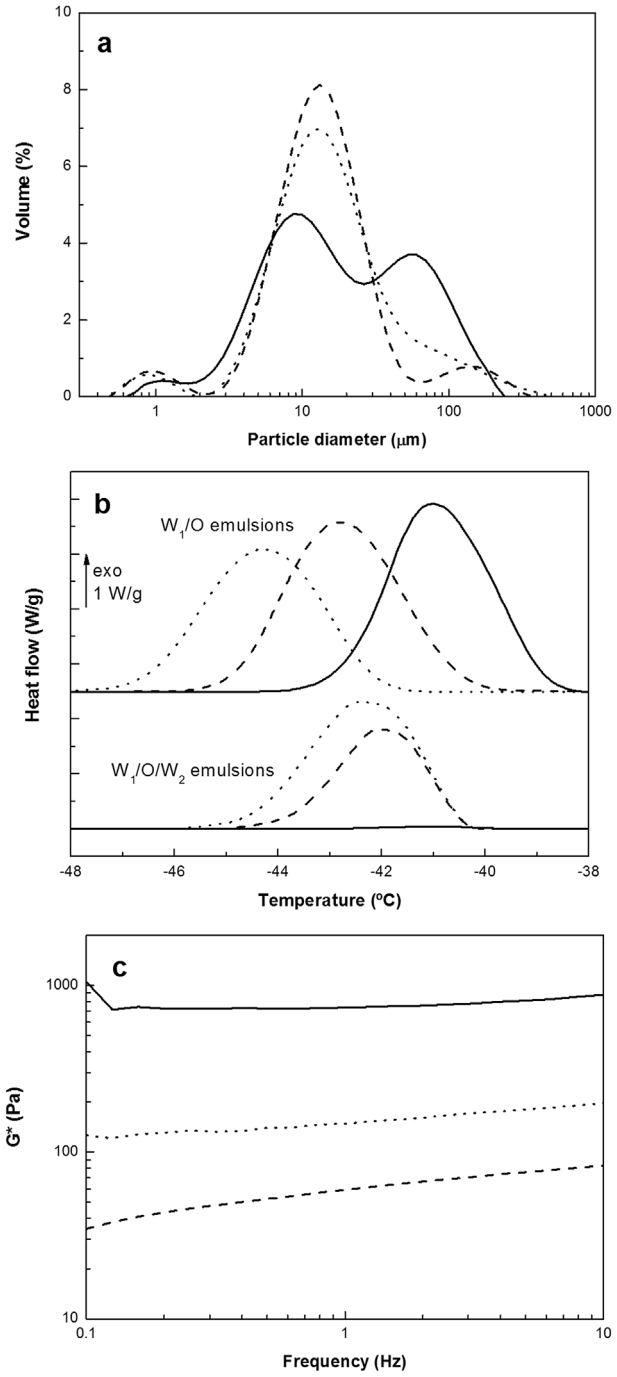

Figure 3. Analysis of emulsions prepared with LT and 1.0% PGPR in lipid phase, with variation of glucose concentration in dispersed aqueous phase: no glucose (——), 4.9% (– – –), and 9.8% (· · · ·). (a) Particle size distribution of $W_1/O/W_2$ emulsions. (b) Exothermic peaks corresponding to freezing of dispersed aqueous phase of W_1/O and $W_1/O/W_2$ emulsions. (c) Oscillatory rheology of $W_1/O/W_2$ emulsions.

exothermic peak area was observed at higher glucose concentration in $W_1/O/W_2$ emulsions; thus, the encapsulation efficiency values increased with the glucose concentration (Table 1). The optical micrographs of $W_1/O/W_2$ emulsions confirm these results, as the absence of glucose led to more aggregation and less W_1 droplets than the other systems (Fig. 4a–c). The generation of an osmotic gradient due to the absence of solutes in dispersed aqueous phase and the presence of milk solutes in continuous aqueous phase may have promoted the release of W_1 droplets during and after the second homogenization stage [2]. Moreover, the lower retention of W_1 droplets at lower glucose concentration can be linked to their larger size (inner d_{43}; Table 1) [2]. In this way, the inner interface area would be reduced and an additional quantity of PGPR would be freed and available to favor partial coalescence by the increase of collision frequency and/or capture efficiency. In turn, the rupture of the outer interfacial film during the partial coalescence process may favor the further loss of dispersed aqueous phase, so that the phenomenon would be auto-induced by a feedback effect. This would explain the very low encapsulation efficiency value observed in the emulsion with no glucose.

On the other hand, the addition of 4.9% glucose in the dispersed aqueous phase of the $W_1/O/W_2$ emulsion, corresponding to isotonic concentration with milk, led to less aggregation and considerably higher encapsulation efficiency than the absence of glucose (Table 1). The equilibration of the osmolalities of both aqueous phases would have prevented the diffusion of water, reducing the PGPR availability to promote partial coalescence. In comparison to the last system, slight microstructural changes were observed with the inclusion of 9.8% glucose in dispersed aqueous phase, surpassing the osmolality of milk and producing a small diminution of the main population at 15 μm with a corresponding increase of number of particles with diameters larger than 40 μm (Fig. 3a). These changes could be attributed to the swelling of globules in the emulsion with higher glucose concentration due to the transport of water from the continuous aqueous phase to the dispersed aqueous phase, as a result of the osmotic unbalance [5, 26]. Although this emulsion presented the highest retention of W_1 droplets, its encapsulation efficiency value did not reach 100% (Table 1), indicating that the release was higher than the incorporation of dispersed aqueous phase. This loss of dispersed aqueous phase is supposed to occur during the second homogenization stage and part of it may have been recovered by the osmotic unbalance after the emulsification process. It should be taken into account that SFC did not show a significant variation in these systems (Table 1); thus, it should not be considered as a potential factor for the observed results.

The variation of glucose concentration in dispersed aqueous phase led to rheological changes in $W_1/O/W_2$ emulsions because of the different microstructures of the

Table 1. Effect of glucose concentration in dispersed aqueous phase on different parameters of $W_1/O/W_2$ emulsions prepared with LT and 1.0% PGPR in lipid phase

Glucose concentration	Outer d_{43} (μm)	Inner d_{43}* (μm)	Encapsulation efficiency (%)	SFC** (%)	tan δ***
No glucose	34.07 ± 3.61^{b}	2.10 ± 0.14^{b}	1.01 ± 0.01^{a}	26.24 ± 0.12^{a}	0.545 ± 0.070^{b}
4.6%	22.91 ± 1.52^{a}	1.79 ± 0.16^{ab}	48.61 ± 0.49^{b}	25.18 ± 1.07^{a}	0.278 ± 0.041^{a}
9.8%	23.58 ± 3.14^{a}	1.52 ± 0.01^{a}	90.22 ± 8.23^{c}	25.02 ± 0.35^{a}	0.253 ± 0.048^{a}

Values are means of two replicates ± SD. Mean values with different letters indicate significant differences between samples ($p < 0.05$).
*Measured on fresh primary W_1/O emulsions.
**SFC in lipid phase of emulsions at 7°C.
***tan δ values at 1 Hz.

systems. The absence of glucose produced the highest G* values (Fig. 3c), attributed to the higher partial coalescence degree. Meanwhile, the addition of 9.8% glucose in dispersed aqueous phase led to higher G* values than 4.9% glucose, because the higher retention of W_1 droplets meant a higher volume fraction of dispersed W_1/O phase and hence the interaction between particles was increased [26]. In addition, it has been reported that the higher rigidity of globules given by their higher content of dispersed aqueous phase leads to more dissipation of mechanical energy into friction, increasing the viscosity of the $W_1/O/W_2$ emulsion [27]; but in the present work, the G* values would be mainly governed by the partial coalescence degree. Furthermore, the emulsions with glucose showed lower tan δ values (i.e., higher elastic character) than the system with no glucose (Table 1). This last result indicates that a higher retention of W_1 droplets tends to favor G' over G", in agreement with a previous work where it was shown that a higher volume fraction of dispersed aqueous phase enhances the elastic character of the $W_1/O/W_2$ emulsion [28].

3.3 Effect of PGPR concentration in $W_1/O/W_2$ emulsions

The particle size distribution of $W_1/O/W_2$ emulsions was also affected by the lipophilic emulsifier concentration in lipid phase. Results indicate that the increase of PGPR content produced an increase of number of particles with diameters larger than 40 μm (Fig. 5a), leading to higher outer d_{43} values (Table 2). A higher PGPR concentration in lipid phase would have increased the partial coalescence promotion because of a higher availability of the surfactant. The exothermic peaks of dispersed aqueous phase in W_1/O and $W_1/O/W_2$ emulsions showed lower freezing temperatures at higher PGPR

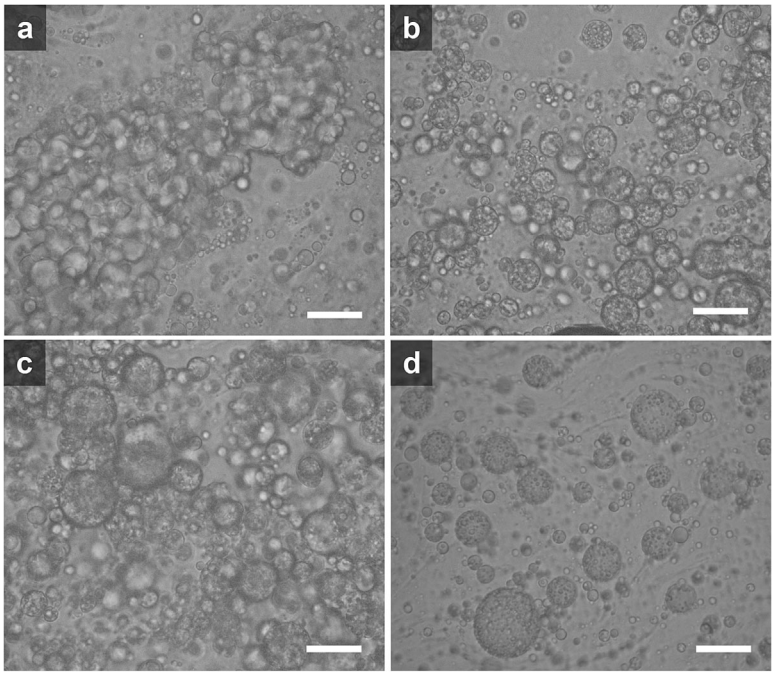

Figure 4. Optical micrographs of $W_1/O/W_2$ emulsions. (a) LT, 1.0% PGPR in lipid phase and no glucose in dispersed aqueous phase. (b) LT, 1.0% PGPR in lipid phase and 4.9% glucose in dispersed aqueous phase. (c) LT, 1.0% PGPR in lipid phase and 9.8% glucose in dispersed aqueous phase. (d) SO, 1.0% PGPR in lipid phase and 4.9% glucose in dispersed aqueous phase. Bar = 20 μm.

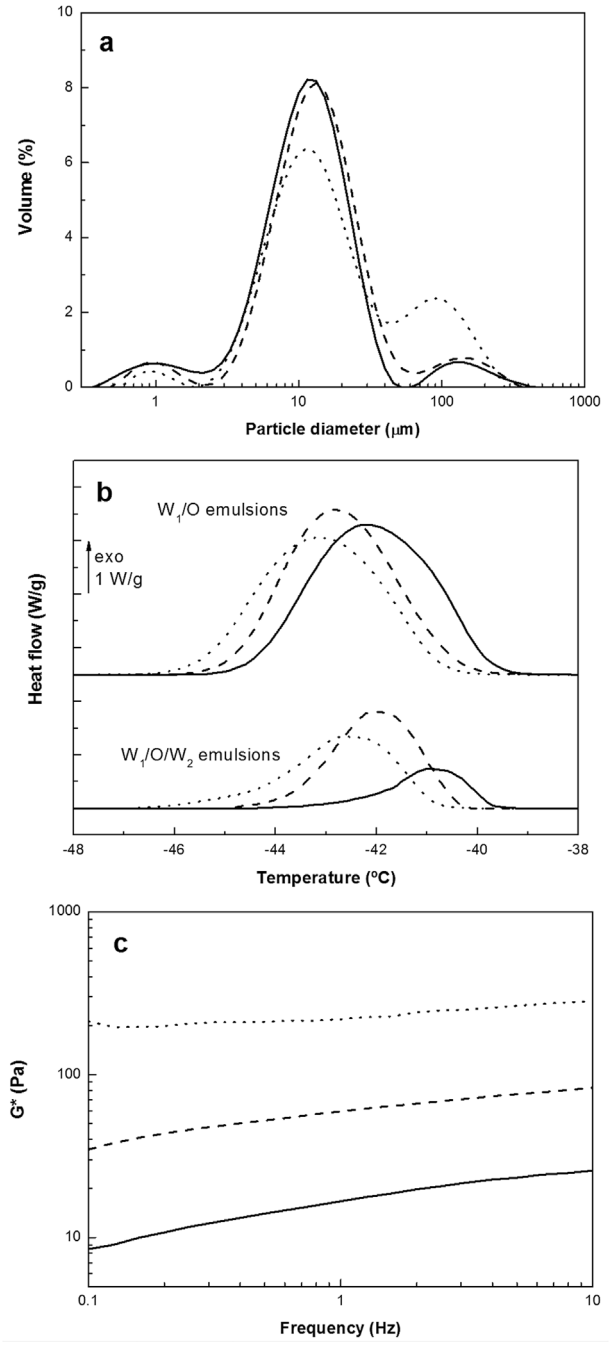

Figure 5. Analysis of emulsions prepared with LT and 4.9% glucose in dispersed aqueous phase, with variation of PGPR concentration in lipid phase: 0.5% (——), 1.0% (– – –), and 2.0% (· · · ·). (a) Particle size distribution of $W_1/O/W_2$ emulsions. (b) Exothermic peaks corresponding to freezing of dispersed aqueous phase of W_1/O and $W_1/O/W_2$ emulsions. (c) Oscillatory rheology of $W_1/O/W_2$ emulsions.

concentration (Fig. 5b), because W_1 droplets size (inner d_{43}) decreased with increasing emulsifier content (Table 2) due to the increased capability to produce interfacial area [18, 20]. Moreover, a smaller exothermic peak area was observed in the $W_1/O/W_2$ emulsion with 0.5% PGPR, so that this system presented a lower encapsulation efficiency value (Table 2). The bigger W_1 droplets produced at that emulsifier content would have been more prone to coalesce and be released toward the continuous aqueous phase during storage [2]. However, the increase of PGPR concentration from 1.0 to 2.0% led to a slight decrease in the encapsulation efficiency value (Table 2), probably because the release of W_1 droplets increased with the partial coalescence degree due to the rupture of the outer interfacial film, as it was previously explained.

The rheological changes produced by the variation of the lipophilic emulsifier concentration in lipid phase were concomitant with the previous microstructure results. The G^* values increased with increasing PGPR concentration (Fig. 5c) because of the increase of partial coalescence degree. Moreover, the lower retention of W_1 droplets in the $W_1/O/W_2$ emulsion with 0.5% PGPR led to a lower volume fraction of dispersed W_1/O phase, giving an additional explanation of the lower G^* values observed in this system. Furthermore, the lower encapsulation efficiency in the last emulsion was confirmed by a higher tan δ value (Table 2).

3.4 Effect of the employed fat in $W_1/O/W_2$ emulsions

The fat composition in the lipid phase was another factor affecting the particle size distribution of $W_1/O/W_2$ emulsions. The control emulsion prepared with SO presented a bimodal distribution (Fig. 6a) and its outer d_{43} value was lower than those given by the other lipid systems (Table 3) due to the absence of partial coalescence (Fig. 4d), as a consequence of the lack of fat crystallization during cold storage (SO crystallized approximately at −55°C according to the DSC measurement of the corresponding emulsion). The employment of LT, BF, and HS produced particles with diameters larger than 40 μm as a result of partial coalescence; and particularly, the emulsion prepared with HS presented a higher outer d_{43} value and hence a higher partial coalescence degree. The microstructural differences given by different fats could be explained by two parallel factors: encapsulation efficiency and SFC. The encapsulation efficiency values followed the order LT > BF > HS (Table 3), confirming that the partial coalescence degree increases with increased release of W_1 droplets at same PGPR concentration in lipid phase. In turn, the variation of encapsulation efficiency values seems to be linked to different W_1 droplets sizes, as it is evidenced by the exothermic peaks (Fig. 6b) and the inner d_{43} values (Table 3). According to these results, HS led to bigger W_1 droplets than the other fats, favoring the release of dispersed aqueous phase [2]. It should be mentioned that the

Table 2. Effect of PGPR concentration in lipid phase on different parameters of $W_1/O/W_2$ emulsions prepared with LT and 4.9% glucose in dispersed aqueous phase

PGPR concentration (%)	Outer d_{43} (μm)	Inner d_{43}* (μm)	Encapsulation efficiency (%)	SFC** (%)	tan δ***
0.5	19.36 ± 0.98^{a}	2.42 ± 0.14^{c}	16.52 ± 0.65^{a}	23.88 ± 1.00^{a}	0.404 ± 0.015^{b}
1.0	22.91 ± 1.52^{b}	1.79 ± 0.16^{b}	48.61 ± 0.49^{c}	25.18 ± 1.07^{a}	0.278 ± 0.041^{a}
2.0	31.01 ± 0.29^{c}	0.72 ± 0.02^{a}	45.92 ± 0.43^{b}	27.35 ± 1.32^{a}	0.276 ± 0.018^{a}

Values are means of two replicates ± SD. Mean values with different letters indicate significant differences between samples ($p < 0.05$).
*Measured on fresh primary W_1/O emulsions.
**SFC in lipid phase of emulsions at 7°C.
***tan δ values at 1 Hz.

encapsulation efficiency of the system prepared with SO was not determined because the exothermic peak of dispersed aqueous phase in the corresponding $W_1/O/W_2$ emulsion was not detected. The complete loss of dispersed aqueous phase during storage was discarded because the presence of W_1 droplets was confirmed by optical microscopy (Fig. 4d). A plausible explanation could be the diffusion and full release of dispersed aqueous phase after freezing of continuous aqueous phase during cooling in the DSC measurement, attributed to the different vapor pressures of ice and undercooled water at the same temperature [21] and the absence of solid fat as a containment barrier.

SFC data provides a complementary explanation of the particle size distribution results. The SFC values clearly followed the order HS > BF > LT at 5–25°C range (Fig. 6c), implying that the higher the SFC the higher the partial coalescence degree, according to the particle size distribution and the outer d_{43} values of $W_1/O/W_2$ emulsions (Fig. 6a; Table 3). In a previous work where O/W emulsions were prepared with the same fats, the SFC of the systems

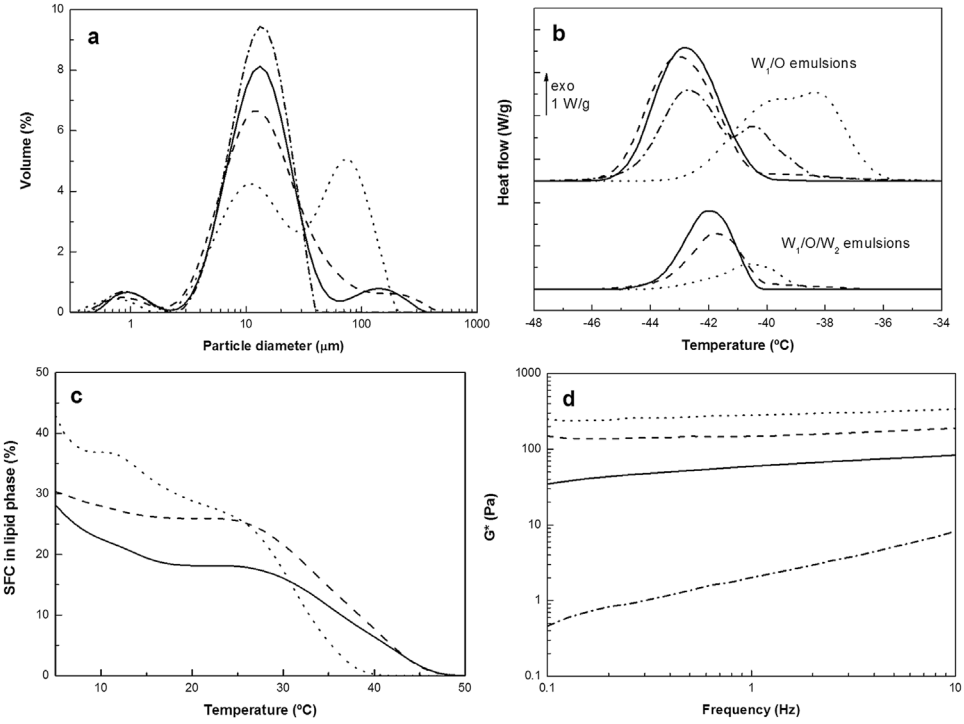

Figure 6. Analysis of emulsions prepared with 1.0% PGPR in lipid phase and 4.9% glucose in dispersed aqueous phase, with variation of the employed fat: LT (——), BF (– – –), HS (· · ·), and SO (– · – ·). (a) Particle size distribution of $W_1/O/W_2$ emulsions. (b) Exothermic peaks corresponding to freezing of dispersed aqueous phase of W_1/O and $W_1/O/W_2$ emulsions. (c) SFC of $W_1/O/W_2$ emulsions. (d) Oscillatory rheology of $W_1/O/W_2$ emulsions.

Table 3. Effect of the employed fat on different parameters of $W_1/O/W_2$ emulsions prepared with 1.0% PGPR in lipid phase and 4.9% glucose in dispersed aqueous phase

Fat	Outer d_{43} (μm)	Inner d_{43}* (μm)	Encapsulation efficiency (%)	SFC** (%)	tan δ***
LT	22.91 ± 1.52^{b}	1.79 ± 0.16^{a}	48.61 ± 0.49^{c}	25.18 ± 1.07^{a}	0.278 ± 0.041^{a}
BF	24.43 ± 2.31^{b}	2.15 ± 0.21^{ab}	35.13 ± 0.46^{b}	29.31 ± 0.97^{b}	0.330 ± 0.032^{a}
HS	41.88 ± 3.49^{c}	4.33 ± 0.54^{c}	16.51 ± 1.70^{a}	38.14 ± 1.62^{c}	0.286 ± 0.063^{a}
SO	12.66 ± 0.08^{a}	2.67 ± 0.03^{b}	–	0	1.240 ± 0.031^{b}

Values are means of two replicates ± SD. Mean values with different letters indicate significant differences between samples ($p < 0.05$).
*Measured on fresh primary W_1/O emulsions.
**SFC in lipid phase of emulsions at 7°C.
***tan δ values at 1 Hz.

presented the same order [14]. Then it was observed that partial coalescence was favored in the cases of BF and HS after application of a temperature cycle, producing enrichment in fat crystals at the interface. It is known that partial coalescence depends on the solid fat/liquid oil ratio in lipid phase, because the fat globules join together when a solid fat crystal from one globule penetrates into the liquid oil portion of another globule [11]. In the present work, partial coalescence was induced by the presence of PGPR in lipid phase; and the capture efficiency may have been increased by a SFC value corresponding to an optimum solid fat/liquid oil ratio. Because HS led to wider SFC ranges during cooling, the $W_1/O/W_2$ emulsion prepared with that fat had higher probabilities of reaching an optimum condition at certain temperature, explaining its higher partial coalescence degree in comparison to the systems prepared with LT and BF. Moreover, the lower encapsulation efficiency observed at higher SFC could be related to a higher release of W_1 droplets through the broken film when the partial coalescence process was favored. Furthermore, PGPR could have different effects on the properties and orientation of fat crystals in different fats, leading to different partial coalescence degrees.

The employment of different fats in lipid phase also had an effect on the rheology of $W_1/O/W_2$ emulsions. The G^* values followed the order HS > BF > LT > SO (Fig. 6d), coinciding with the order of SFC at 5–25°C range (Fig. 6c). This result was attributed to the different partial coalescence degrees of the emulsions, which followed the same order according to the microstructure study. Moreover, a higher SFC value also increases the solid-like characteristic of the aggregated globules, leading to a more rigid three-dimensional network and, thus, higher G^* values [13, 14]. With regard to the tan δ values, LT, BF, and HS did not present significant differences despite their different encapsulation efficiency values (Table 3), probably because a lower retention of W_1 droplets was compensated by a higher SFC, which would enhance the elastic character of the emulsions. On the other hand, the emulsion prepared with SO showed the highest tan δ value, because in this case the totally liquid composition of the lipid phase would favor G" over G', enhancing the viscous character of the system.

4 Conclusions

The microstructure and rheology data allow concluding that PGPR played a role on the partial coalescence process in $W_1/O/W_2$ emulsions prepared with skimmed milk. One explanation could be the displacement of dairy proteins by the lipophilic emulsifier at the outer interface, reducing the repulsive interaction between globules and increasing collision frequency. Another effect of PGPR could be the modification of the properties and orientation of fat crystals, increasing capture efficiency. The release of W_1 droplets as a consequence of an osmotic unbalance enhanced the partial coalescence process, probably due to the increased availability of PGPR to induce the phenomenon. Therefore, the addition of glucose in dispersed aqueous phase increased the encapsulation efficiency and decreased the partial coalescence degree as a result of the equilibration of the osmotic pressures in the inner and outer aqueous phases. At relatively low PGPR concentration the encapsulation efficiency decreased, attributed to the generation and loss of bigger W_1 droplets; and an excess of PGPR favored the partial coalescence process due to the increase of the lipophilic emulsifier availability. The use of different fats led to varied partial coalescence degrees; the phenomenon was enhanced at lower encapsulation efficiency and higher SFC. Moreover, partial coalescence seemed to produce further release of W_1 droplets due to the rupture of the outer interfacial film, resulting in a feedback effect that maximized aggregation of fat globules and loss of dispersed aqueous phase at certain conditions. Overall, it was found a correlation between inner osmolality, encapsulation efficiency and inhibition of partial coalescence. Therefore, the obtained results are useful to understand and control the factors leading to partial coalescence in $W_1/O/W_2$ emulsions prepared with dairy proteins and including PGPR and solid fat crystals in the lipid phase.

The authors acknowledge the financial support from Universidad Nacional de Quilmes (Program I + D PUNQ 53/1037) and they thank CALSA (Lanús, Argentina) for the provision of the fats and PGPR.

The authors declare the absence of conflicts of interest. M. P. Pérez is fellow and J. R. Wagner and A. L. Márquez are researchers of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

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