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PARTIAL COALESCENCE IN CREAM-LIKE EMULSIONS PREPARED WITH ALTERNATIVE FATS: EFFECT OF CONTROLLED STIRRING AND TEMPERATURE CYCLES

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KEYWORDS
Controlled stirring, emulsion, microstructure, partial coalescence, rheology, temperature cycle

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ABSTRACT

The objective was to study the partial coalescence as a consequence of controlled stirring and temperature cycles in oil-in-water emulsions prepared with skimmed cow milk and nondairy fats: low trans vegetable fat (LT), bovine fat (BF), partially hydrogenated soybean oil (HS) and sunflower oil as control. The partial coalescence rates (%/min) of the stirred emulsions were LT, 89.8 ± 4.3; BF, 29.7 ± 3.1; HS, 23.8 ± 3.1. This result was attributed to the lower solid fat content (%) of emulsion LT (19.2 ± 1.2 versus 25.9 ± 1.1 in BF and 56.8 ± 1.1 in HS). A temperature cycle with partial melting of fats led to hardening of texture in emulsions BF and HS due to partial coalescence, favored by the recrystallization of the fat at the interface. Emulsion LT did not show changes after the same treatment, probably because its lower solid fat content did not favor partial coalescence in the absence of mechanical work.

PRACTICAL APPLICATIONS

This article deals with the formulation and characterization of cream-like emulsions prepared with skimmed cow milk and different fats as an alternative lipid phase instead of dairy fat. The partial coalescence process was analyzed after application of controlled stirring (where the phenomenon is desired) and temperature cycles (where it must be avoided). This research can be useful as a starting point for the elaboration of a healthier product than dairy cream without losing its characteristic rheological behavior during whipping/stirring. This work also points to control some desired and undesired properties by the selection of the lipid phase. Promising results were obtained using a particular vegetable fat with low content of trans fatty acids; this may open the door for further investigations in order to obtain a healthy edible cream with favorable response to whipping/stirring and good stability against thermal fluctuations.

INTRODUCTION

Dairy creams are oil-in-water (o/w) emulsions whose dispersed lipid phase is partially composed of solid fat. When whipping or stirring is applied, these emulsions eventually increase their thickness if they contain an adequate quantity of lipid phase and solid fat (Vanapalli and Coupland 2001; Fredrick *et al.* 2010). This phenomenon involving rheological changes is the result of a partial coalescence process, by which fat globules form a rigid three-dimensional network (Boode and Walstra 1993; Boode *et al.* 1993; Goff 1997). Because the mechanical work increases the collision

frequency, the fat globules join together when a solid fat crystal from one globule penetrates into the liquid oil portion of another globule, so the partial coalescence rate (PCR) depends not only on the flow conditions but also on the solid fat/liquid oil ratio in the lipid phase (Rousseau 2000; Fredrick *et al.* 2010). The thickness is increased because the effective volume of the formed aggregates is higher than the sum of the volumes of the individual globules, because of the presence of continuous aqueous phase trapped within them (McClements 1999). In previous works from our group, this behavior was imitated by developing o/w emulsions prepared with soybean milk and a lipid phase composed by sunflower oil (SO) and a high melting fraction of dairy fat (Márquez *et al.* 2005a,b) or vegetable fat with low content of trans fatty acids (Márquez and Wagner 2012).

As a quality parameter, dairy creams should stay as a fluid liquid until the moment of its consumption. Thus, it is necessary to minimize the external factors that could lead to the destabilization of the system (Mutoh *et al.* 2001). When the conditions of stages like transport or storage interrupt the cold chain, the liquid cream could be solidified as a consequence of the thermal fluctuation. Other authors have reported that this rheological change is observed when the creams are re-cooled after an increase of temperature in which the solid fat is partially melted, while the systems keep their liquid texture if the fat is totally melted (Boode *et al.* 1991; Goff 1997). According to Fredrick *et al.* (2010), the process by which the creams increase their thickness after heat treatments is partial coalescence, which is favored by a higher capture efficiency, i.e., how frequently two globules join together after a collision. This process occurs even at the absence of mechanical work (low collision frequency) because the new solid fat distribution inside the globules favors the phenomenon. Previously, we reproduced this behavior on o/w emulsions prepared with soybean milk and a lipid phase composed by SO and high melting fraction of dairy fat, which were subjected to different temperature cycles (Márquez *et al.* 2005c).

Because the traditional dairy cream contains a high proportion of saturated fats, it is recommended to limit its consumption because of risk of cardiovascular disease (Huth and Park 2012). The employment of an alternative vegetable lipid phase could be a solution to this problem. Moreover, the selected vegetable fat should contain a low quantity of trans fatty acids, which are also associated to the risk of heart diseases (Mozaffarian *et al.* 2006). The selection of the lipid phase could also be a key to control partial coalescence, favoring the phenomenon when it is desired and reducing it when it must be avoided.

The objective of the present work was to study the partial coalescence process in o/w emulsions prepared with skimmed cow milk and different alternative fats as lipid phase, in order to analyze the effect of the dispersed phase on the degree and rate of the phenomenon as a consequence of controlled stirring. The effects of temperature cycles were also studied. The changes in the microstructure and rheology of the systems were analyzed. Therefore, the partial coalescence process was analyzed in emulsions with different lipid phase to traditional dairy cream at conditions when the phenomenon is desired (controlled stirring) and undesired (temperature cycles).

MATERIALS AND METHODS

Materials

This study used skimmed cow milk powder (S.A. La Sibila, Rosario, Argentina), distilled water, xanthan gum (Parafarm, Buenos Aires, Argentina), low trans vegetable fat (LT), refined bovine fat (BF), partially hydrogenated soybean oil (HS), refined SO (Molino Cañuelas SACIFIA, Cañuelas, Argentina) and commercial dairy cream (Mastellone Hnos. S.A., General Rodríguez, Argentina). LT, BF and HS were provided by CALSA (Lanús, Argentina). SO was used as control and the dairy cream was not used for comparative reasons but as an example of the partial coalescence process in a commercial system.

The composition of the milk powder was proteins, 29.2% w/w (determined by micro Kjeldahl); humidity, 7.5%; and ashes, 7.5% w/w. The melting points (determined by differential scanning calorimetry [DSC] as the temperature of melting peak) of the fats were LT, 45C; BF, 45C; and HS, 42C. LT was obtained by interesterification of natural fatty acids and fully hydrogenated fatty acids from vegetable oil (70% cotton oil and 30% soybean oil, approximately). BF corresponds to refined tallow obtained from bovine tissue. The fatty acid composition of the fats is shown in Table 1. The fat content of the dairy cream was 42% w/v, according to the product label.

Preparation of Emulsions

All emulsions were prepared with reconstituted cow milk (containing 9.0% w/w skimmed milk powder and 0.2% w/w xanthan gum as stabilizer) as the continuous aqueous phase and 40% w/w of dispersed lipid phase using different fats (LT, GB, AS and SO). Emulsions (sample weight, 100 g) were homogenized using a rotor-stator homogenizer Ultraturrax T-25 (IKA-Labortechnik, Staufen, Germany) with a S25-20NK-18G rotor (IKA-Labortechnik) at 24,000 rpm for 1 min. Previously, the fats were melted and the aqueous phases were heated (both at 80C) to avoid crystallization of the fat and generate pasteurization conditions during homogenization. Samples were immediately cooled

TABLE 1. FATTY ACID COMPOSITION (%) OF DIFFERENT FATS

	Sample			
Fatty acid	LT	BF	HS	SO
Myristic ($C_{14:0}$)	0.55	3.30	0.09	–
Myristoleic ($C_{14:1t}$)	–	0.28	–	–
Myristoleic ($C_{14:1c}$)	0.04	0.76	–	–
Pentadecanoic ($C_{15:0}$)	–	0.72	–	–
Pentadecanoic ($C_{15:1c}$)	–	0.24	–	–
Palmitic ($C_{16:0}$)	19.99	25.50	10.76	8.31
Palmitoleic ($C_{16:1t}$)	0.33	0.42	0.11	–
Palmitoleic ($C_{16:1c}$)	0.16	2.82	–	–
Margaric ($C_{17:0}$)	–	1.36	–	–
Margaroleic ($C_{17:1t}$)	–	0.11	–	–
Margaroleic ($C_{17:1c}$)	–	0.53	–	–
Stearic ($C_{18:0}$)	28.31	21.70	17.00	2.52
Elaidic ($C_{18:1t}$)	0.19	3.97	39.90	–
Oleic ($C_{18:1c}$)	12.62	34.84	29.46	24.17
Linoleic ($C_{18:2t,t}$)	–	0.21	1.10	–
Linoleic ($C_{18:2c,t}$)	0.13	–	0.10	–
Linoleic ($C_{18:2t,c}$)	0.17	–	0.70	–
Linoleic ($C_{18:2c,c}$)	36.07	2.10	0.38	64.83
Linolenic ($C_{18:3}$)	1.42	0.55	0.07	0.17
Arachidic ($C_{20:0}$)	–	0.48	–	–
Behenic ($C_{22:0}$)	–	–	0.30	–

The composition of LT, BF and HS, determined by gas chromatography, was provided by CALSA (Lanús, Argentina) and the data corresponding to SO were estimated from the information given by the product label and bibliography (Chowdhury *et al.* 2007).
BF, bovine fat; HS, hydrogenated soybean oil; LT, low trans vegetable fat; SO, sunflower oil.

in cold water. The emulsions were stored at 7C for 1 day before the corresponding treatments and characterizations.

Controlled Magnetic Stirring

Emulsions were stirred with a magnetic stirrer at a speed of 150 rpm and room temperature (21 ± 1C). Samples were stirred until the stirring rod stopped as a consequence of thickness increase in the system (Márquez *et al.* 2005b). For the emulsion prepared with SO, a stirring time of 30 min was determined, as this system did not show thickness increase.

Temperature Cycles

Each emulsion was subjected to two different temperature cycles. In cycle 1, after initial storage at 7C for 1 day, emulsions were heated slightly below the melting point of each fat (40C for alternative fats and 30C for dairy cream) for 30 min and then re-cooled in new storage at 7C for 1 day. In cycle 2, same steps were repeated but heating was performed above the melting point of the fats (55C for alternative fats and 40C for dairy cream).

Optical Microscopy

Micrographs were obtained with an optical microscope operating at 400× magnification and using an adapted digital camera (Canon A570 IS, Shah Alam, 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., Workcestershire, 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, Malvern Instruments Ltd.) 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 (Anton *et al.* 2002) were performed.

The d_{43} values obtained before and after controlled stirring were used to calculate the partial coalescence degree (PCD) and apparent PCR as follows:

$$PCD = \frac{d_f - d_0}{d_0} \times 100 \quad (1)$$

$$PCR = \frac{PCD}{t} \quad (2)$$

where d_0 and d_f are the d_{43} values of the initial and stirred emulsions, respectively, and t is the stirring time required to stop the stirring rod.

DSC

Thermograms of the emulsions were obtained using a MDSC Q-200 instrument (TA Instruments, New Castle, DE). Samples were enclosed in hermetically sealed aluminum pans and then cooled until 4C at 10C/min (with an isotherm at that temperature for 10 min) and heated until 70C at 5C/min.

Solid fat content (SFC) of the emulsions was calculated using a corrected method for SFC estimation by DSC, recently proposed by our group. In this method, melting energy was transformed into melted mass, where a linear correlation between melting enthalpy and melting point of different triglycerides was used (Márquez *et al.* 2013). Because in the present work emulsions were only cooled to 4C to prevent freezing of the aqueous phase, a full thermogram to analyze crystallization of the fat phase was not obtained. Therefore, the method for SFC estimation was adapted using the following equation:

$$SFC = \frac{\int_{T}^{T_f} m dT}{M} \times 100 \quad (3)$$

where m is the melted mass as a function of the temperature (T), T_f is the final temperature of melting, and M is the total sample mass weighted in the pan.

Rheology

Oscillatory rheology of the emulsions was studied using an AR-G2 rheometer (TA Instruments) with a cone-and-plate geometry (gap, 55 μm; cone diameter, 40 mm; cone angle, 2°). Temperature (21C) was controlled with a water bath (Julabo ACW100, Julabo Labortechnik, Seelbach, Germany) associated with the rheometer. Experimental data for emulsions with different treatments 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 (one-way ANOVA) and test of least significant difference ($P < 0.05$) using the statistical program Statgraphics Plus 5.1 (Statgraphics Corporation, Princeton, NJ). A two-way ANOVA was performed in order to detect an interaction between alternative fats and treatments using the program Origin 7.0 (OriginLab Corporation, Northampton, MA). For the statistical analysis of rheological data, G′ and G″ values were previously transformed into their decimal logarithm. Two or three independent replicates were measured at least two times in each experiment.

RESULTS AND DISCUSSION

Effect of Controlled Stirring

All emulsions showed a liquid texture before controlled stirring was applied, whereas the systems containing solid fat increased their thickness after being stirred. This behavior was attributed to the formation of aggregates because of the partial coalescence of fat globules induced by the mechanical work (Boode and Walstra 1993; Boode *et al.* 1993; Goff 1997). Figure 1 shows optical micrographs of emulsions prepared with different fats before and after stirring. In all cases, it was observed that the fat/oil globules were generally dispersed as individual particles at the initial condition (Fig. 1A–E). After the application of mechanical work, aggregation produced by partial coalescence was observed in emulsions containing alternative solid fats (LT, BF and HS; Fig. 1F–H). With regard to the emulsion prepared with SO, the oil droplets increased their size as a consequence of "true" coalescence induced by stirring (Fig. 1I); in this case, partial coalescence or thickness increase were not observed because this sample did not contain solid fat. On the other hand, the commercial dairy cream did show the partial coalescence phenomenon after stirring (Fig. 1J), as it is the usual characteristic of this food emulsion (Vanapalli and Coupland 2001).

The particle size distributions of the emulsions can be observed in Fig. 2. Before controlled stirring, the systems prepared with every alternative fat showed a bimodal distribution, including a main population with mode value slightly below 10 μm and a secondary population with mode at 1 μm (Fig. 2A–D). In contrast, the commercial dairy cream initially showed a monomodal distribution with a defined population with mode at 4 μm (Fig. 2E). This difference could be attributed to the different homogenization method used in the industry for the production of dairy cream. Moreover, the type of alternative fat seemed to have an effect on the particle size of the emulsions before stirring, according to their initial d_{43} values (Table 2). It should be considered that in all cases, determinations made in the presence of SDS as deflocculating agent did not show decrease in particle size (data not shown), indicating the absence of stable flocs generated by bridging flocculation under the measurement conditions. Furthermore, no significant changes were observed in the particle size of the emulsions after 1 month of refrigerated storage (data not shown), demonstrating the absence of coalescence and/or flocculation. Creaming was neither observed during that storage time, attributed to the presence of 0.2% xanthan gum as stabilizer in the continuous phase, as it was previously demonstrated in stability studies with cream-like emulsions prepared with soybean milk (Márquez *et al.* 2005a).

Controlled stirring produced notorious changes in the microstructure of the emulsions. In all cases it was observed the appearance of a new population of higher particle sizes at the expense of the main initial population (Fig. 2). This increase in particle size was attributed to the partial coalescence process previously observed in the optical micrographs of emulsions prepared with alternative solid fats (Fig. 1F–H) as well as in dairy cream (Fig. 1J). The result corresponding to SO should be interpreted as "true" coalescence, according to the phenomenon observed by optical microscopy in this sample (Fig. 1I). These microstructural changes were also manifested by a significant increase in the d_{43} values after stirring in all cases (Table 2). The emulsion prepared with LT showed the highest PCD (Table 3), indicating that it presented the highest increase of particle size. The required stirring times in order to produce thickness increase were the following: LT, 7 min; BF, 9.5 min; HS,

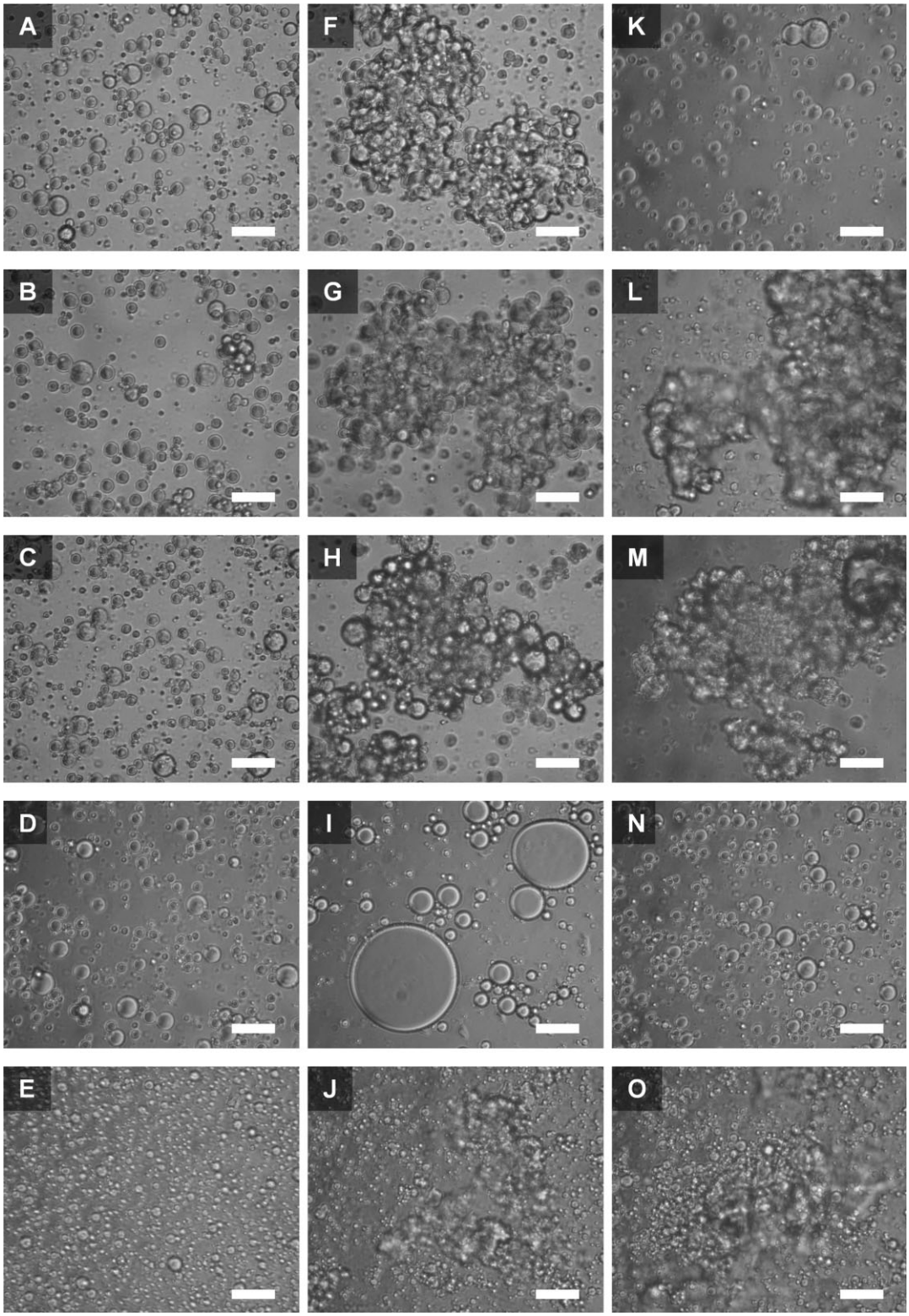

FIG. 1. OPTICAL MICROGRAPHS OF O/W EMULSIONS WITH DIFFERENT ALTERNATIVE FATS AS LIPID PHASE (1:10 DILUTION), BEFORE AND AFTER CONTROLLED STIRRING AND TEMPERATURE CYCLE

Initial samples: (A) low trans vegetable fat (LT); (B) bovine fat (BF); (C) hydrogenated soybean oil (HS); (D) sunflower oil (SO); (E) dairy cream. Stirred samples: (F) LT; (G) BF; (H) HS; (I) SO; (J) dairy cream. Cycle 1 samples: (K) LT; (L) BF; (M) HS; (N) SO; (O) dairy cream. Bar = 20 μm.

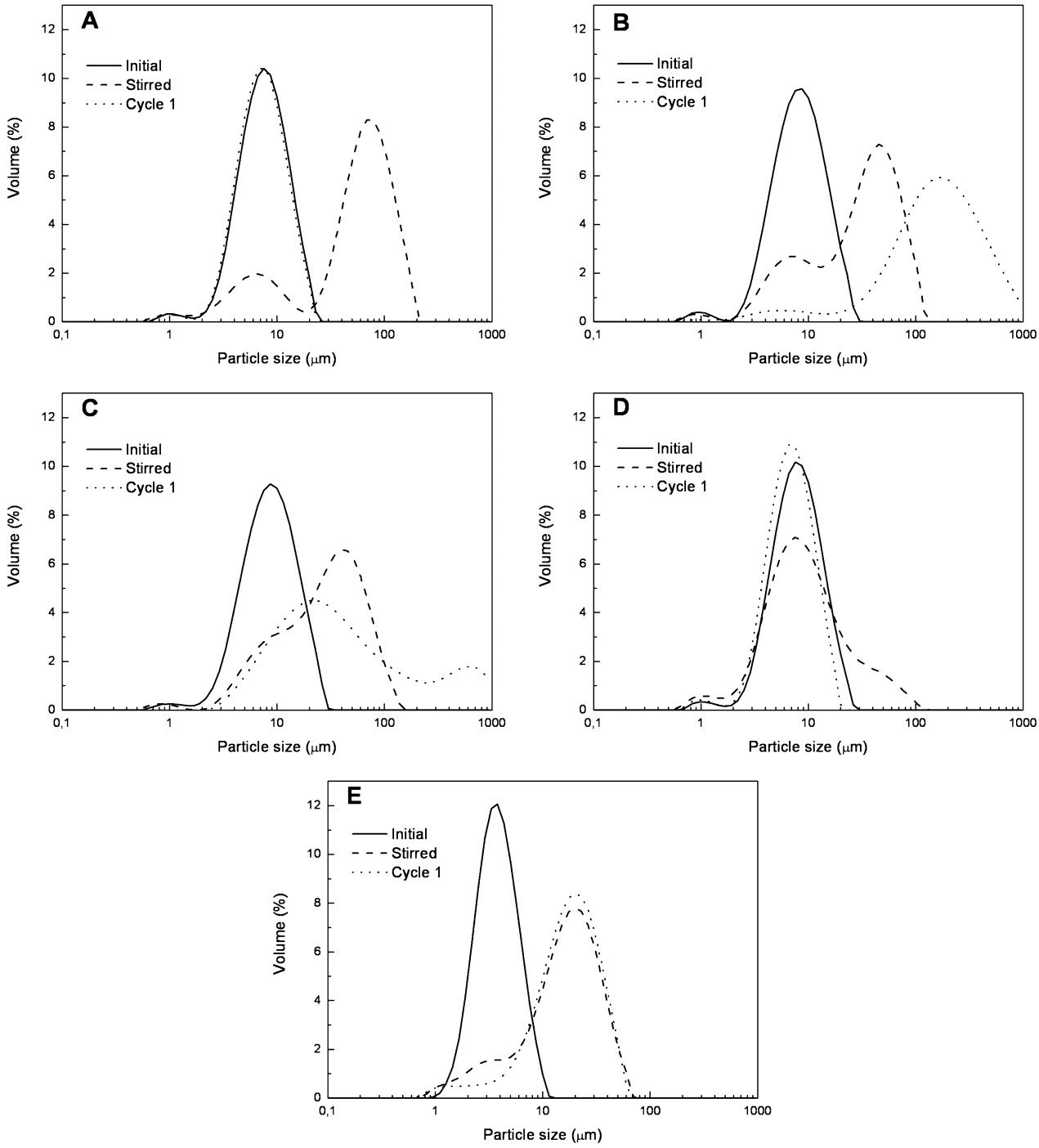

FIG. 2. PARTICLE SIZE DISTRIBUTION OF O/W EMULSIONS WITH DIFFERENT ALTERNATIVE FATS AS LIPID PHASE, BEFORE AND AFTER CONTROLLED STIRRING AND TEMPERATURE CYCLE
(A) Low trans vegetable fat; (B) bovine fat; (C) hydrogenated soybean oil; (D) sunflower oil; (E) dairy cream.

10.5 min. Thus, the emulsion LT presented the highest PCD and the lowest stirring time, so it naturally showed the highest PCR (Table 3). These results suggest that the emulsion LT presented the highest capture efficiency in order to produce partial coalescence when two globules collide each other. Fredrick *et al.* (2010) pointed out that partial coalescence requires the presence of both solid and liquid parts in the dispersed lipid phase, because this phenomenon occurs

TABLE 2. MEAN PARTICLE DIAMETER (D_{43}) OF O/W EMULSIONS WITH DIFFERENT ALTERNATIVE FATS AS LIPID PHASE, BEFORE AND AFTER CONTROLLED STIRRING AND TEMPERATURE CYCLES

	d_{43} (μm)			
Sample	Initial	Stirred	Cycle 1	Cycle 2
LT	7.66 ± 0.12^{b}■	55.85 ± 2.60●	7.77 ± 0.34■	7.41 ± 0.04■
BF	8.50 ± 0.18^{c}■	32.43 ± 2.11●	183.16 ± 24.92▲	8.39 ± 0.02■
HS	9.05 ± 0.34^{d}■	31.58 ± 1.79●	118.69 ± 6.12▲	9.27 ± 0.06■
SO	6.74 ± 0.06^{a}■	13.82 ± 0.56●	6.75 ± 0.04■	6.82 ± 0.03■
Dairy cream	3.80 ± 0.03■	17.67 ± 0.25●	18.32 ± 0.23▲	3.79 ± 0.04■

Values are means of three replicates ± standard deviation. Mean values with different letters indicate significant differences between different alternative fats in emulsions at initial condition ($P < 0.05$). Mean values with different symbols indicate significant differences between different treatments for the same sample ($P < 0.05$).

BF, bovine fat; HS, hydrogenated soybean oil; LT, low trans vegetable fat; SO, sunflower oil.

when a solid fat crystal from one globule penetrates into the liquid oil portion of another through the surrounding thin film. The emulsion LT could have a solid fat/liquid oil ratio closer to the optimum than the other systems at the stirring temperature, which would explain its higher capture efficiency. This optimum value refers only to SFC and could be different for each system, because partial coalescence is also governed by other factors like particle size or location and size of fat crystals (Rousseau 2000; Fredrick *et al.* 2010). At the stirring temperature, the emulsion LT presented the lowest SFC among the systems prepared with alternative solid fats (Table 3). Even though the SFC in the lipid phase of the emulsion LT was lower than 20%, i.e., containing more liquid oil than solid fat, most of the solid fat would be located at the interface, because the interfacial free energy of the system is the lowest when the crystals are situated in that zone (Walstra 2003; Fredrick *et al.* 2010). Increasing SFC leads to decreasing space for liquid oil to interact with a solid fat crystal from another globule; thus, above a certain SFC the capture efficiency would be reduced. Although the optimum SFC may differ for each system, the results suggest that the higher SFC values of emulsions BF and HS were responsible for their lower PCR values in comparison with LT. It should also be mentioned that the SFC of all samples did not show a significant change after stirring (data not shown). The PCD values would also be linked to the capture efficiency; according to McClements (1999), the structures formed by aggregation of particles are more open when the attraction or interaction between particles is stronger. When globules join together rapidly, they are not free to roll round each other; thus, the formation of aggregates with close packing is prevented. Because our calculation of PCD depends not only on the number of partially coalesced globules but also on the quantity of continuous phase trapped within the aggregates, a more open packing would lead to bigger structures and consequently to higher PCD values. Therefore, the higher PCD of the emulsion LT could be explained by the higher capture efficiency presented by this system.

TABLE 3. PARTIAL COALESCENCE DEGREE (PCD), PARTIAL COALESCENCE RATE (PCR) AND SOLID FAT CONTENT (SFC) OF O/W EMULSIONS WITH DIFFERENT ALTERNATIVE FATS AS LIPID PHASE

Sample	PCD (%)	PCR (%/min)	SFC* in lipid phase (%)
LT	628.8 ± 30.1^{a}	89.8 ± 4.3^{a}	19.2 ± 1.2^{a}
BF	281.7 ± 29.9^{b}	29.7 ± 3.1^{b}	25.9 ± 1.1^{b}
HS	250.0 ± 32.5^{b}	23.8 ± 3.1^{b}	56.8 ± 1.1^{c}

PCD and PCR values are means of three replicates ± standard deviation (SD); SFC values are means of two replicates ± SD. Mean values with different letters indicate significant differences between samples ($P < 0.05$).

*SFC values correspond to initial samples at 21C.

BF, bovine fat; HS, hydrogenated soybean oil; LT, low trans vegetable fat.

The emulsions BF and HS presented higher initial particle sizes than LT (Table 2). Larger globules allow the formation of larger fat crystals (Lopez *et al.* 2002), leading to greater crystal protrusions through the interface (Giermanska *et al.* 2007) and larger pore sizes in the crystal network (Fredrick *et al.* 2010). The greater the protrusion distance the higher the PCR, and the larger the pore size the higher the availability of liquid oil as a lubricating agent for the creation of a permanent junction (Fredrick *et al.* 2010). Therefore, a higher globule size should lead to higher capture efficiency. However, the emulsion LT presented the highest PCR even though its initial particle size was lower than BF and HS; this result reinforces the theory that SFC was a main factor for capture efficiency.

Figure 3 shows the viscoelasticity study of the emulsions, where results obtained before and after controlled stirring can be observed. In all cases, G′ (Fig. 3A) was higher than G″ (Fig. 3B), giving values of tan δ (G″/G′) lower than 1; this indicates that the systems have higher elastic than viscous characteristics. Before stirring, the G′ and G″ values were higher in emulsions with higher SFC. The presence of higher quantity of solid fat at the employed volume fraction of dispersed lipid phase (40%) would have a significant effect on the rheology of the systems, increasing the

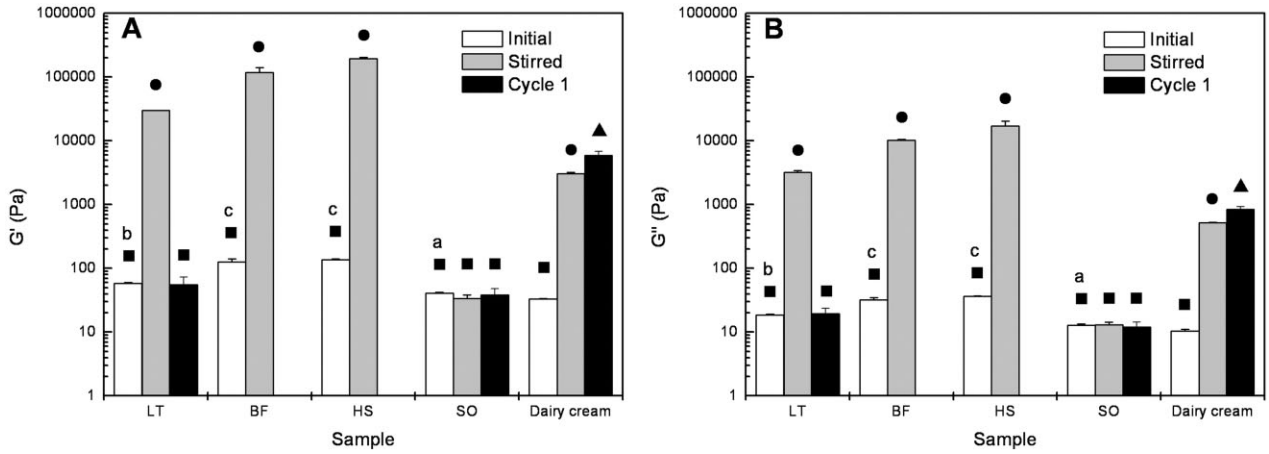

FIG. 3. OSCILLATORY RHEOLOGY OF O/W EMULSIONS WITH DIFFERENT ALTERNATIVE FATS AS LIPID PHASE, BEFORE AND AFTER CONTROLLED STIRRING AND TEMPERATURE CYCLE

(A) Elastic modulus (G′). (B) Viscous modulus (G″). The experiment could not be performed for bovine fat and hydrogenated soybean oil creams after cycle 1 because of the hard texture of the samples. Values are means of two replicates and error bars indicate standard deviation. Mean values with different letters indicate significant differences between different alternative fats in emulsions at initial condition ($P < 0.05$). Mean values with different symbols indicate significant differences between different treatments for the same sample ($P < 0.05$).

viscoelastic parameters because of the interaction of globules with higher solid-like characteristics (Márquez and Wagner 2012). Controlled stirring produced an increase of the G′ and G″ values in all the emulsions containing solid fat, as a consequence of the partial coalescence process. Although the stirred emulsion LT showed a higher PCD (Table 3), it did not present higher G′ and G″ values than stirred BF and HS, probably because the higher SFC of the last samples had a more important effect on the magnitude of the viscoelastic parameters as a consequence of the formation of a more rigid three-dimensional network.

Effect of Temperature Cycles

Emulsions subjected to temperature cycle 1 were heated slightly below the melting point of the corresponding fat phase and then re-cooled to 7C. This treatment produced hardening of the texture of the systems, except in the emulsion LT, which stayed as a liquid cream. These rheological changes were attributed to the partial coalescence process favored by changes in the crystallization of the lipid phase of the emulsions (Boode *et al.* 1991). Optical micrographs of the emulsions subjected to cycle 1 can be observed in Fig. 1. The emulsion LT did not show appreciable changes in the microstructure after application of cycle 1 (Fig. 1K), explaining the absence of rheological changes in this system. On the other hand, emulsions BF and HS did show important aggregation of fat globules by partial coalescence after cycle 1 (Fig. 1L,M). The heat treatment did not produce microstructural changes in the emulsion SO because of the absence of solid fat (Fig. 1N), while the dairy cream presented the typical partial coalescence phenomenon generally observed when it is subjected to these temperature variations (Fig. 1O). Meanwhile, after application of temperature cycle 2, which included full melting of the fat phase, in all cases hardening of the texture nor changes in the microstructure of the emulsions according to the analysis by optical microscopy (data not shown) was not observed.

The effect of temperature cycle 1 on the particle size distribution of the emulsions can be observed in Fig. 2. After application of this cycle, the emulsion LT did not show changes in its microstructure (Fig. 2A), confirming the result previously observed by optical microscopy. Moreover, the d_{43} value of this sample did not present a significant variation when cycle 1 was applied (Table 2). On the other hand, the emulsions BF and HS subjected to cycle 1 showed a new main population of higher particle sizes, with the almost complete disappearance of the initial main population (Fig. 2B,C). In these two systems, the increase in particle size as a consequence of cycle 1 was much more important than the effect of controlled stirring, as can be observed by the comparison of the corresponding d_{43} values (Table 2). The emulsion SO naturally did not show increase of particle size after cycle 1 (Fig. 2D) while the dairy cream presented a new population of bigger particles after the treatment (Fig. 2E), although this effect was more moderated than in emulsions BF and HS. It should be taken into account that the systems did not show microstructural changes immediately after the heating stage (data not shown); thus, in cases where increase of particle size was observed, the partial coalescence phenomenon occurred

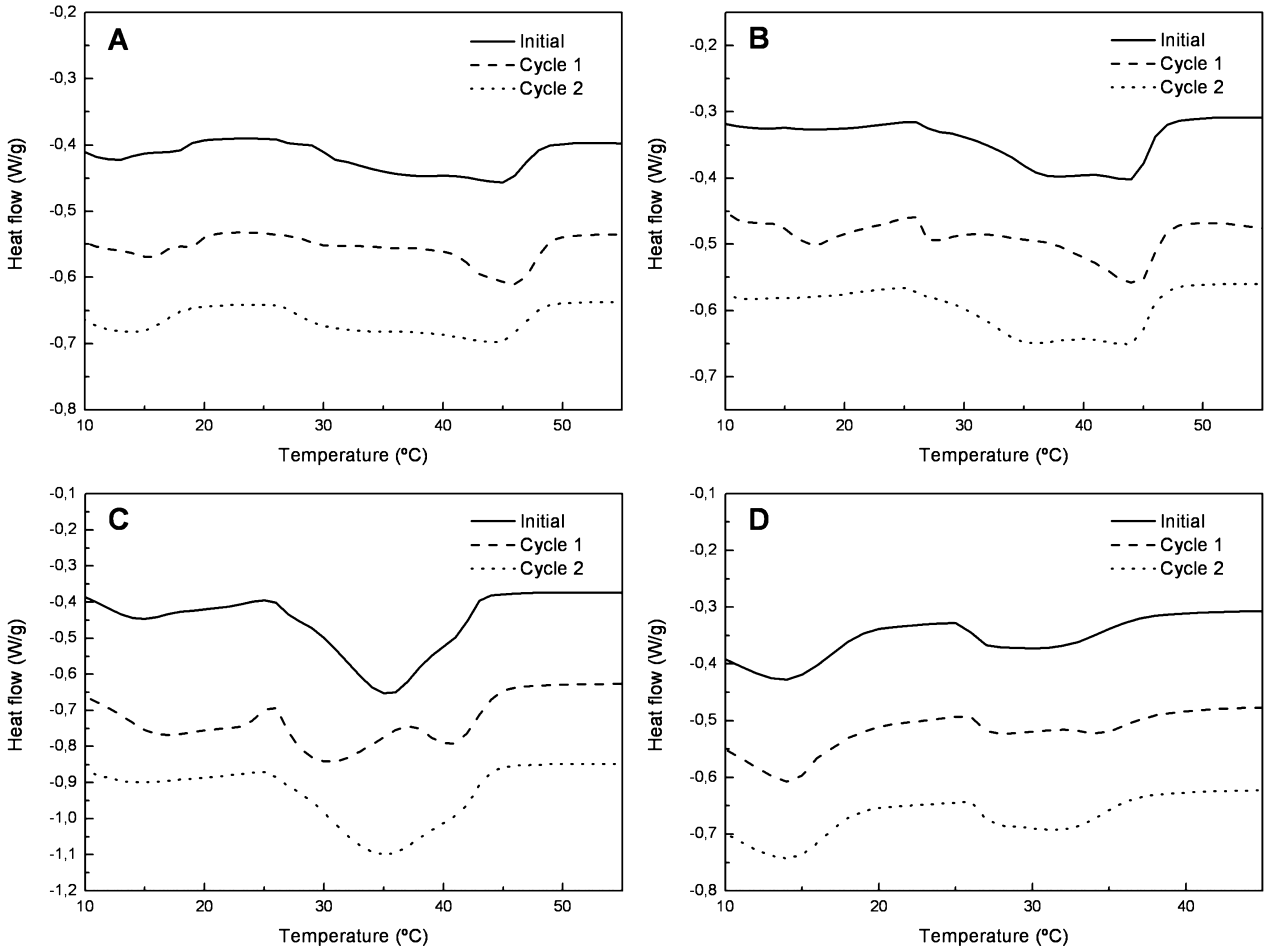

FIG. 4. DIFFERENTIAL SCANNING CALORIMETRY THERMOGRAMS OF O/W EMULSIONS WITH DIFFERENT ALTERNATIVE FATS AS LIPID PHASE, BEFORE AND AFTER TEMPERATURE CYCLES
(A) Low trans vegetable fat; (B) bovine fat; (C) hydrogenated soybean oil; (D) dairy cream.

during cold storage. With regard to temperature cycle 2, in all cases no significant differences were observed in the d_{43} values in comparison with the initial condition (Table 2).

The statistical analysis by two-way ANOVA indicated a significant interaction between the alternative fats (LT, BF and HS) and the treatments (stirring and temperature cycles) for the d_{43} values ($P < 0.05$). This result shows that the response to the treatments was subjected to the type of fat employed.

The DSC thermograms show the effect of both temperature cycles on the crystallization of the lipid phase of the emulsions (Fig. 4). In all cases, temperature cycle 1 produced notorious changes in the crystallization of the fat after re-cooling, observing an enrichment in higher melting point crystals. Because in this treatment the emulsions were heated slightly below the melting point of corresponding fat, full melting of the lipid phase was prevented; thus, a number of high melting point crystals would have remained. These remaining crystals, no longer subjected to a continuous network, could move freely toward the interface, in order to reach the lowest interfacial free energy (Boode *et al.* 1991; Walstra 2003). In this way, during the re-cooling stage, the remaining crystals would act as nuclei for the new crystallization, leading to enrichment in crystal fats at the interface. As the number and probably the size of crystals situated at the interface increased (Boode *et al.* 1991), more crystals would be available to penetrate the interface and with a further distance (Fredrick *et al.* 2010). This would lead to a favorable situation for the occurrence of partial coalescence when two or more globules collide each other, in this case because of the increased capture efficiency instead of collision frequency. On the other hand, when temperature cycle 2 was applied, the DSC thermograms were similar to those corresponding to the initial condition. In this case, the lipid phase was fully melted, so the absence of remaining crystals after heating did not allow

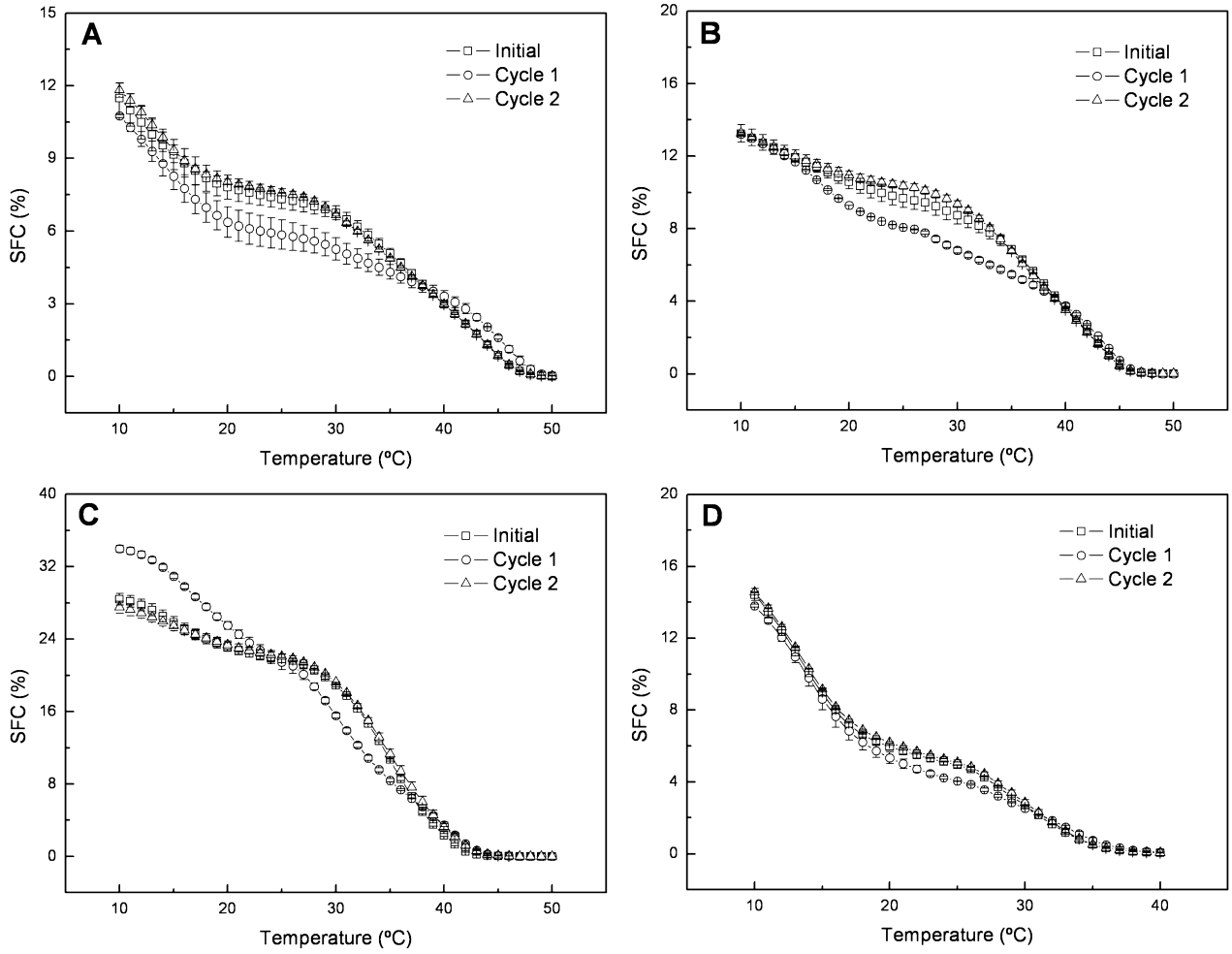

FIG. 5. SOLID FAT CONTENT (SFC) AS A FUNCTION OF THE TEMPERATURE OF O/W EMULSIONS WITH DIFFERENT ALTERNATIVE FATS AS LIPID PHASE, BEFORE AND AFTER TEMPERATURE CYCLES
(A) Low trans vegetable fat; (B) bovine fat; (C) hydrogenated soybean oil; (D) dairy cream. Values are means of two replicates and error bars indicate standard deviation.

a richer crystallization at the interface during re-cooling. Thus, the new crystallization did not show appreciable differences with the initial samples, because they were cooled starting from the same totally liquid lipid phase.

Although the application of temperature cycle 1 is supposed to favor the partial coalescence process, this phenomenon did not occur in every emulsion containing solid fat. As it was previously showed, partial coalescence was not detected in emulsion LT after cycle 1, as it did occur in the other systems; one possible explanation could be related to the SFC of the emulsions. Figure 5 shows the variation of the SFC values with the temperature, comparing initial samples with those subjected to temperature cycles. In all cases, cycle 1 produced lower SFC values at intermediate temperatures, but the results were similar or even higher than initial samples at lower temperatures. Emulsion LT showed the lowest SFC values at lower temperatures (Fig. 5A); this could explain the absence of partial coalescence in this system. Although the type of crystallization of emulsion LT after cycle 1 was similar to the other systems (Fig. 4A), the quantity and size of fat crystals at the interface would not be enough to produce thickness increase without the need of mechanical work. Even though the emulsion LT was the most prone to partial coalescence when controlled stirring was applied, the phenomenon occurred at a determined temperature increasing the collision frequency between globules, so the circumstances were different. However, when this system was stirred having been applied cycle 1, the required stirring time was reduced (~30%), indicating that the heat treatment certainly favored partial coalescence and, thus, a more rapid thickness increase. With regard to emulsions BF and HS, as well as the dairy cream,

they all showed partial coalescence and texture changes after cycle 1 but presenting different SFC values at lower temperatures (Fig. 5B–D). Nevertheless, when heated emulsions were re-cooled their SFC increased until reaching the lowest temperature, so each system may have reached an optimum SFC value at certain point where partial coalescence was highly favored (Fredrick *et al.* 2010). This behavior was not observed after application of temperature cycle 2, as the SFC values of all emulsions were similar to those corresponding to initial samples at the whole temperature range. Thus, the absence of partial coalescence after this treatment can be explained by the absence of changes in the type of crystallization in the lipid phase.

The viscoelasticity study allows the comparison between the effects of controlled stirring and temperature cycle 1 on the rheology of the emulsions (Fig. 3). The emulsion LT did not show a significant change of the G′ and G″ values after cycle 1, confirming the absence of partial coalescence in this system. Thus, the employment of LT as lipid phase seems to give higher stability against temperature cycles without losing the property of increasing thickness with stirring. Although we were not able to obtain G′ and G″ values after cycle 1 for emulsions BF and HS because of their hard texture (it was not possible to reach the required gap between cone and plate), the effect of the heat treatment on the rheology of these systems was clearly more abrupt than the result of controlled stirring, as it was also manifested by the analysis of the microstructure (Fig. 2B,C). Finally, the emulsion SO did not show significant rheological changes with the treatments because it did not contain solid fat, and the dairy cream showed slightly higher increase of the G′ and G″ values after cycle 1 than after controlled stirring.

CONCLUSIONS

The results obtained in this work show that the partial coalescence process can be reproduced in o/w emulsions prepared with skimmed milk and alternative fats. The emulsion prepared with LT was more prone to partial coalescence during controlled stirring than those prepared with BF and HS, having the former system a lower SFC value. However, the emulsion LT did not show partial coalescence when it was subjected to a temperature cycle with heating slightly below the melting point of the fat, while the phenomenon did occur in emulsions BF and HS when the same treatment was applied, producing strong hardening of the texture. The results obtained for LT were promising because the system prepared with this fat showed to be prone to partial coalescence when the process is desired (whipping or stirring) and more stable to the same phenomenon when it needs to be avoided (thermal fluctuations). If we sum the vegetable origin and low content of trans fatty acids of the fat, it can be concluded that LT is a good potential substitute of dairy fat for the formulation of cream-like emulsions, in terms of both functionality and healthiness.

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