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ORIGINAL ARTICLE

The Impact of Polyoxyethylene Sorbitan Surfactants in the Microstructure and Rheological Behaviour of Emulsions Made With Melted Fat From Cupuassu (*Theobroma grandiflorum*)

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Abstract Cupuassu fat is a good candidate for partial substitution of cocoa butter in many products, including emulsions. However, for such use it is necessary to know the characteristics of the products prepared with cupuassu fat. Therefore, the main goal of this work is to characterize emulsions prepared with cupuassu fat using the surfactants Tween® 60, Tween® 80 and Tween® 85 as emulsifiers. The emulsions were prepared at 43 °C with addition of 0.5 or 1.5 % (w/v) of surfactant and compared with an emulsion without surfactant. All emulsions were analysed by conductivity, stability, pH, optical microscopy, rheology and oxidative stability. It was verified that the emulsions prepared with Tween® 60 and Tween® 80 have higher stability, smaller droplet size and higher apparent viscosity. Also, these properties are positively influenced by the concentration of the surfactant. On the other hand, emulsions prepared with Tween 85 or without surfactant reached unsatisfactory results. The rheological behaviour of the emulsions was adequately described by both Herschel-Bulkley and Mizhari-Berki models revealing pseudoplastic character. These emulsions also present strong gel behaviour, with storage modulus higher than loss modulus. In conclusion, cupuassu fat can be used as oil phase for emulsions products and this characterization helps to understand their behaviour in order to increase their use in food industry.

Introduction

Cupuassu (*Theobroma grandiflorum*), also known as "cupuaçu", which belongs to the same genus as cocoa (*Theobroma cacao*), one of the most important among the native Brazilian Amazonian fruits, stands out economically mainly by the characteristics of flavour, aroma and possibilities for domestic and agro-industrial use of its pulp. Its seeds has a high fat content (62 %) [1], comprised of triacylglycerols with a balanced composition of saturated (53–58 %) and unsaturated (39–45 %) fatty acids [1–3], which gives the product a low melting point (starting at approximately 30 °C), because of its high content of monounsaturated fatty acids, mainly oleic acid, and the appearance of a soft solid that quickly melts on contact with skin [3].

Cupuassu fat has been the subject of some studies in last few years due to the similarities between its physicochemical [4–6] and biological characteristics [7] with those of cocoa butter. In addition, it is used popularly in the production of candies and confectionery in the northern and northeastern regions of Brazil, where the *Theobroma* species was originally found, being considered as an excellent raw material for utilization in the food industry, and it is also a good candidate to partially substitute cocoa butter in chocolate products [8, 9]. Recent interest in this fat also include its application in products such as chocolate bars, chocolate powder, breads and cakes [1, 10].

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However, even with a great potential of use in many different products, this fat is currently mostly used in the cosmetic industry, generating surpluses [11].

It is well known that oils and fats are the main material in the production of emulsions; they are largely applied in different products, and their properties can be controlled by varying the components added and the production process [12, 13]. Recently, emulsions produced using vegetable oils and fats have been studied, where stability and rheological parameters are the main criteria for monitoring quality standards [13–15] and also to characterize particle–particle interactions [16]. The flow properties are among the most important physical attributes of an emulsion, both technically and aesthetically. Hence, the ability to measure, adjust, and, if possible, predict such properties through rheology is very important [17, 18]. Moreover, the Rancimat, an automated technique to quickly determine the oxidative stability of oils and fats has also been used in the study of lipid systems, as emulsions to provide good results regarding the life cycle of such products [19–21]. It is important to consider that kinetically stable emulsions are formed using amphiphilic compounds [22–24] such as the polysorbates, that have the capacity to self-assemble into membranous structures in the form of bilayers.

Polysorbates containing 20 units of oxyethylene are hydrophilic non-ionic surfactants and they are used widely as emulsifying agents in the preparation of oil-in-water emulsions usually to impart certain desirable qualities to products, whose function is simply that of an efficient surfactant that maintains emulsion stability, due to the simultaneous presence of lipophilic and hydrophilic parts in their chemical structure [13, 25]. Mostly known as "Tween" (registered trademark of Croda International PLC), these surfactants have applications that include the area of biosciences, pharmaceutical, food and cosmetics, and they are rather non-toxic and possess an extremely compatible set of physical properties that allow for widespread use along with other surfactants [26–28].

Since emulsions are found in many products, it is important to understand their physical, rheological and microscopic properties in order to choose the best surfactants and process conditions and also the appropriate equipment to work with this type of colloidal system. For this purpose, determinations of the droplet size and rheology are probably the most important way to characterise them [13, 28–30]. In addition, these criteria depend on several parameters like the type and concentration of the surfactant and oil phase; in consequence, this influences other properties of emulsions such as stability, texture, appearance, taste, shelf life, etc. [31].

Considering there are few studies in the literature about cupuassu fat as a source for the production of emulsions, the present study aimed to evaluate the effect of type and concentration of polyoxyethylene sorbitan surfactants on rheology, microstructure and stability parameters of emulsions produced with melted cupuassu fat stabilised by Tween® 60 (polyoxyethylene (20) sorbitan monostearate), Tween® 80 (polyoxyethylene (20) sorbitan monooleate), or Tween® 85 (polyoxyethylene (20) sorbitan trioleate) surfactants, developed under different emulsification conditions and analysed in temperature settings exceeding 40 °C, avoiding the presence of microcrystals or solidification. Surfactant influences on rheological properties and the stability of the proposed formulations were investigated, as well as the possible emulsifying ability of this fat, relating this information to the microstructure of these emulsions.

Experimental

Materials

The oil phase was crude cupuassu fat (provided by FECAT—Federação das Cooperativas da Agricultura Familiar do Sul do Pará, Brazil). Distilled water with 1 % (w/v) NaCl (Vetec, Brazil) was the aqueous phase. Tween® 80 (Vetec, Brazil), Tween® 60, and Tween® 85 (Sigma-Aldrich, St. Louis, USA) were used as surfactants with known Hydrophilic-Lipophilic Balance (HLB): 15.0, 14.9, and 11, respectively.

Emulsion Formation

Preliminary tests were carried out on emulsions containing 20, 40, 50, 60, 70 and 80 % (v/v) oil phase and concentrations of 0.5 % and 1.5 % (w/v) surfactant. Unsatisfactory results were observed when less than 80 % (v/v) oil phase was used, whereas more stable emulsions were found with 80 % (v/v) oil phase plus 0.5 % and 1.5 % (w/v) surfactant, so only these emulsions were studied.

Oil phase composition samples, shown on Table 1, were prepared considering different concentrations of 80 % (v/v) total oily phase in 200 mL total volume. Figure 1 illustrates the scheme for production of emulsions. The oil phase was made by dispersing the surfactant in fat that was previously melted in water bath (Q215 M; Quimis Aparelhos Cientificos, Brazil) at 43 ± 2 °C. Sample preparation was also performed at the same temperature. To avoid thermal shock, the aqueous phase was equally heated. Ingredients were then mechanically stirred (9000 rpm) in a 250 mL glass beaker using a L4RT Silverson homogeniser (Silverson Machines Ltd, UK) for 5 min.

One control emulsion without any surfactant added was formulated to investigate the fat's characteristics and

Table 1 Composition of the oil phase of the samples prepared under different concentrations of the oily phase 80 vol. % in 200 mL total volume

Sample	Oil phase composition	
	Cupuassu fat (vol. %)	Surfactant (w/v)
C80	100	0
T60A	99.5	0.5
T80A		
T85A		
T60B	98.5	1.5
T80B		
T85B		

C80 emulsion without surfactant (control), *T60A and T60B* emulsions with Tween® 60, *T80A and T80B* emulsions with Tween® 80, *T85A and T85B* emulsions with Tween® 85

The letters "A" and "B" indicate 0.5 and 1.5 % (w/v) surfactant, respectively

emulsifying capacity without adjuvants. All assays were performed in triplicate.

Characterisation of Emulsion Type

The emulsions were prepared with an aqueous solution containing 1 % (w/v) NaCl in order to identify the emulsion type (water in oil—W/O; or oil in water—O/W) through electrical conductivity measurements (Gehaka CG 2500, São Paulo, Brazil). High conductivity values (more than 0.1 mS/cm) indicate that water is the continuous phase and oil is the dispersed phase in an O/W emulsion, while for a W/O emulsion, the conductivity values will be low (few μS/cm) [13, 32].

Microscopic Analysis and Droplet Size Distribution of Emulsion

In order to investigate the droplet size distribution (DSD) and the effect of shear rate on the internal microstructure of the samples, a Zeiss Axio Observer D1 inverted light microscope was used (Zeiss Vision GmbH, Germany) with 640× magnification. For this, emulsion was carefully deposited in a uniform gap of 1 mm made on a microscope slide (76 mm × 26 mm) and a coverslip (18 mm × 18 mm). Images captured with the AxioCam were analysed by AxioVision software (version 4.8.2). DSD analysis was performed using an adapted methodology from Züge *et al.* [13], where five micrographs were captured for each treatment and the droplets size was measured along the entire length of the image in order to measure 100 random drops, totalling 500 droplets for each condition. The Tukey test was applied to test for significant differences ($p \leq 0.05$) between treatments and droplet size.

Thermal Stress and Centrifugation Test

According to the preliminary tests, after about 1.0 h of rest, all samples solidified and showed no phase separation or release of exudates; as a result, the emulsion stability was determined by thermal stress through the evaluation of phase separation of 10 mL transferred to conical graduated tubes maintained in a water bath at 43 ± 2 °C (Q215 M; Quimis Aparelhos Cientificos, Brazil). Samples were evaluated after 1, 2, 4, and 6 h [33].

For the centrifuge stability test, an Excelsa® II Centrifuge (FANEM®, Brazil) was used at 1000, 2500, and 3500 rpm (139, 873, 1711g, respectively). A DMA 35 portable densimeter (Anton Paar GmbH, Austria) was used for measuring the fat and NaCl solution densities, data required to calculate critical osmotic pressure using an adapted method from Denkov et al. [34]., by the following equation:

$$P_{\mathrm{OSM}}^{\mathrm{CR}} = \Delta \rho g_{\mathrm{k}} \int_{0}^{H_{\mathrm{k}}} \Phi(z) \mathrm{d}z = \Delta \rho g_{\mathrm{k}} (V_{\mathrm{TOT}} - V_{\mathrm{R}})/A_{\mathrm{TT}} \qquad (1)$$

where: $\Delta \rho$ is the difference in the mass densities of the oil and water phases; g_{k} is the centrifugal acceleration, $\Phi(z)$ is the local volume fraction of oil in the cream (**z** is a co-

Fig. 1 Graphic scheme representing cupuassu fat and the production process of emulsions in a glass beaker at 43 °C with the aid of a water bath and a stirrer at 9000 rpm
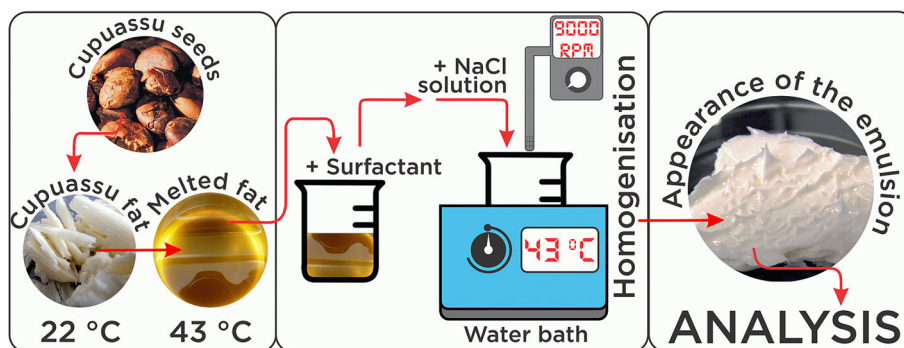
ordinate parallel to the centrifugal field); V_{TOT} is the total volume of oil in the emulsion, V_R is the volume of released aqueous phase in the tube; A_{TT} is the cross-sectional area of the test tube.

Oxidative Stability Index

Oxidative stability index (OSI), also called as induction period (IP) or induction time was evaluated in a Metrohm Rancimat model 743 (Herisau, Switzerland), with 3 ± 0.1 g of sample, 20 L/h air flow, and a temperature of 120 °C, following the American Oil Chemists' Society Official Method Cd 12b-92 [35]. IP was registered by Rancimat 743 PC Software 1.1 (Metrohm). Based on IP data, a possible antioxidant efficiency of the surfactants added can be calculated through protection factor (PF), determined by Eq. 2: [19, 20]

$$\text{Protection factor (PF)} = \text{Induction period of sample}/ \text{induction period of control sample} \quad (2)$$

Apparent pH

Each sample was dispersed in distilled water (10 % w/v) at 25 °C, followed by measurement with a digital pH meter (Model PG1800 Gehaka) previously calibrated with buffer solutions of pH 4.0 and 7.0. The pH of cupuassu fat (CF) was obtained by direct measurement, inserting the electrode into the melted sample at 30 ± 2 °C. It was not possible to measure the pH of the emulsion C80 (control) because it does not mix completely in distilled water. The final result corresponds to the mean of six.

Rheological Analysis and Modelling of Flow Behaviour

Rheological measurements of emulsions and the cupuassu fat were performed in a Haake Mars II (Thermo Electron GmbH, Germany) rheometer connected to a thermostatic bath (Haake K15), thermo-circulator water unit (Haake DC5B3) and a Peltier temperature control (Haake TC 81). A sensor cone-plate with a diameter of 60 mm and a cone angle of 2° was used. Sampling was carried out in triplicate at 45 °C, and the emulsions remained in the rheometer for 5 min prior to analysis. The following measurements were performed: (1) flow curve ($\dot{\gamma} = 0.1$–500 s^{-1}, $t = 300$ s, acquisition of 100 points); (2) dynamic stress sweep ($\tau = 0.1$–100 Pa, $\omega = 1$ Hz) and subsequent dynamic frequency sweep ($\omega = 0.01$–10 Hz, $\tau = 1.0$ Pa); and iii) temperature sweep ($T = 20$–60 °C and 60–20 °C, 2 °C/min, $t = 1200$ s, $\omega = 1$ Hz, $\tau = 1.0$ Pa). Temperature sweeps were performed at a constant tension of 0.5 % in the Linear Viscoelastic Region (LVR). The viscoelastic characteristics of storage modulus (G') and loss modulus (G") were determined during the heat-cool (20–60 °C) cycle. Data collection was performed using RheoWin 3 software (Haake). Graphics and analysis data were generated with the aid of Origin 6.0 software (OriginLab Corporation, MA, USA).

Experimental data obtained from flow curve of emulsions were fitted according to the Herschel-Bulkley (Eq. 3) and Mizrahi-Berk (Eq. 4) rheological models to obtain the rheological and statistical parameters used to assess the adequacy of the flow curves obtained. The equations for the rheological models are defined as:

$$\tau = \tau_{0H} + (\dot{\gamma})_H^n - \text{Herschel - Bulkley (H)} \quad (3)$$

$$\tau^{0.5} = K_{0m} + (K_M)_M^n - \text{Mizrahi - Berk (M)} \quad (4)$$

where: τ is the shear stress (Pa); τ_{0H} is the yield stress (Pa); $\dot{\gamma}$ is the shear rate (s^{-1}), K_H and K_M are the consistency index (Pa.s^n); K_{0M} is the square root of the initial yield stress (Pa); n_H and n_M are the rates of flow behavior models (dimensionless).

Statistical Analysis

Data were analysed by analysis of variance (ANOVA) with significance defined as $p \leq 0.05$. Significant differences between means were determined by Tukey's test at the same significance level with the aid of Statistica 10.0 software (StatSoft Inc.).

Results and Discussion

Emulsion Type

Emulsions containing Tween® 60 and Tween® 80 surfactant presented as O/W, with lower conductivity values (between 400 and 900 μS/cm), while those with Tween® 85 and no surfactant were W/O type (conductivity values between 0.3 and 300 mS/cm).

According to Bancroft's rule, "the phase in which the stabilizing agent is more soluble will be the continuous phase", because during coalescence, the greater availability of surfactant will counteract the stabilizing action of the increased surface tension gradient at the thin film between the drops [28]. Furthermore, by using the HLB concept, the values for Tween® 60 (14.9), Tween® 80 (15.0) and Tween® 85 (11.0) are in the usual range that promote the formation of O/W emulsions (8–18) [27, 36]. Nevertheless, it is noted that Tween® 85 does not produce the usual type of O/W emulsion for several reasons including the high temperature adopted in the emulsification process (43 °C). The high temperature may have led to an unexpected

Fig. 2 Stability test by centrifugal (**a**), and critical osmotic pressure (**b**) of emulsions with 80 % (v/v) oil phase and addition of 0.5 % or 1.5 % (w/v) Tween® 60, Tween® 80 or Tween® 85. g_k = gravitational acceleration
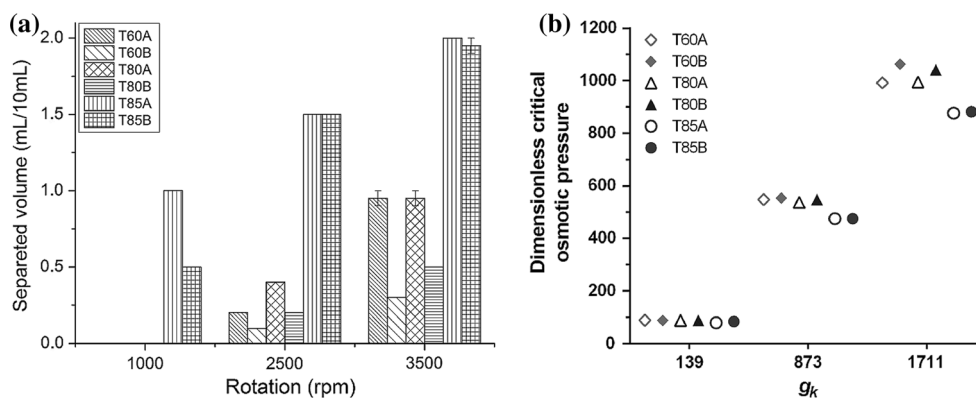
inversion [13] as occurs in the phase inversion temperature (PIT) technique that involves a transitional inversion induced by changing factors that affect the HLB of the surfactants, such as temperature, salt concentration and pH [31, 37–39]. In addition O/W macroemulsions are stable below the PIT, while W/O macroemulsions are stable above the PIT; furthermore, as a rule of thumb the best O/W emulsion stability is achieved 20–50 °C below the PIT. However, the finest dispersed phase is achieved close to the PIT. Consequently, the ideal emulsification procedure involves preparing the O/W emulsion just below the PIT and cooling rapidly [39]; meantime, it was not possible to cool the samples to prevent crystallization of fat and subsequent solidification of the emulsions.

In addition, the higher the HLB of the surfactant, the better for O/W emulsion formation [36]; since Tween® 85 has the lowest HLB of the three surfactants, there was a decrease in the chance of producing a O/W emulsion since polysorbates become increasingly lipophilic with increasing temperature.

It is noteworthy that polyoxyethylene non-ionic surfactants are sensitive to temperature variations; and become lipophilic with increasing temperature due to dehydration of the polyoxyethylene chains [38, 40]. Although considered hydrophilic, systems stabilised by non-ionic surfactants or mixtures thereof often have a characteristic PIT, varying with a range of experimental factors including the amount and nature of the oil present and the nature of the surfactant(s) present [37].

Stability of Emulsions

After the thermal stress test (43 ± 2 °C), only the control emulsion (C80) showed a significant volume of the separated aqueous layer (approximately 1.2 mL) and oil drops on the surface only remained intact for 2.0 h. Emulsions containing 0.5 % (T85A) and 1.5 % (T85B) Tween® 85 showed an almost immeasurable separate aqueous phase (approximately 0.02/10 mL) and oil droplets on the

surface; however, such instability is common in fresh emulsions [28, 41]. On the other hand, all samples containing Tween® 60 and Tween® 80 did not show phase separation during the 6-h heat stress test; this can be explained because cupuassu fat has high water absorption capacity (about 240 %), assigned to H-bonds between water molecules and phytosterols, which aid in the stability of emulsions [3].

Figure 2 shows the results for a centrifuge stability test (A), and critical osmotic pressure (B) of six emulsions containing Tween® 60, Tween® 80 or Tween® 85. Signs of instability were checked by centrifugal test (Fig. 2a) by the increased mobility of the particles as a result of increased gravity. It was found that there was phase separation in all samples except C80, which solidified during the first centrifugation cycle (1000 rpm); therefore, the test could not be applied qualitatively to this emulsion.

The surfactant molecules form a layer around the droplets by adsorbing at the oil–water interface, which prevents coalescence. Nevertheless, Tween® 85 that have large sizes of both hydrophilic and lipophilic moieties enabling the incorporation of greater amounts of oil and water [42] probably produced films (membranes) with bigger interfacial area than the other emulsions, since the emulsions T85A and T85B exhibited the greatest amount of separated aqueous phase (approximately 2.0 mL/10 mL) and therefore less stability. Furthermore, such emulsions presented lower values of critical osmotic pressure (Fig. 2b), indicating that small forces are able to cause separation/coalescence, because only these emulsions showed phase separation at 139 g (1000 rpm).

The osmotic pressure of an emulsion is defined as the pressure difference that should be imposed on the emulsion to maintain equilibrium with its bulk continuous phase (the aqueous phase for oil-in-water emulsions) if the latter is separated by a semipermeable membrane [43]. In this case, the critical pressure for film rupture depends on drop size. In fact, what matters for stability is the size (area) of the emulsion films rather than the drop size itself. Larger films

are less stable, because the probability of an unstable spot formation (nucleus of film rupture) increases with the film area, causing thermodynamic instability [34, 44]. There is also the possibility that the surface fat crystals may have imbibed into the film between the drops, which would result in a point of weakness in the surface layer and cause coalescence [45, 46], and the fact that cupuassu fat shows a lower crystallisation time.

The emulsions T60B and T80A showed much lower separated aqueous phase (between 0.30 ± 0.01 and $0.95 \pm 0.05/10$ mL, respectively), confirming higher stability than those containing Tween® 85. These enhancing effects are probably attributable to the high solubilisation capacity of polysorbates, which are assumed to form large interfacial surfactant film between inner and outer phases in the emulsion [42]. In addition to surfactant moleculaes contributing to the stability of emulsions, another reason is that fat particles (such as crystals) that assist in the stabilisation of emulsions, which can be structured at the interface of the emulsion droplets, providing a physical barrier to coalescence [47]. Moreover, the wettability of crystals in the interface, interfacial rheology of the film, and the particle microstructure (morphology and polymorphism) may also influence the stability of emulsions containing fat crystals [45].

Thus, the presence of fat crystals may stabilise or destabilise the emulsion droplets depending on whether they are intraglobular or are in the continuous phase. If present in the continuous phase, fat crystals contact the interface and adsorb onto the droplet surface, potentially stabilising the dispersion. As part of the dispersed phase, lipids in a state of partial crystallisation can substantially increase the emulsion destabilisation. During processing and/or storage, intraglobular fat (e.g. cream) may solidify to form crystals that can protrude through the interface, leading to droplet coalescence [45, 46].

As explained by Dickinson [23], the main role of the emulsifier is to adsorb at the surface of the freshly formed fine droplets and thus prevent them from coalescing with their neighbours to form larger droplets. By having affinity to both oily and aqueous phases, the surfactants improved the stability of emulsions because they form a highly viscous and stiff interfacial film, which tends to retard the drainage rate of the film and increases the tensile strength, thereby promoting stability [45].

Table 2 shows the results of induction period (IP), protection factor (PF) and apparent pH values. It is noted that cupuassu fat (CF) reached the high induction time of 44.58 h, while all of the emulsions had lower values for IP, because of the other ingredients (especially water) in their composition. Emulsions with higher proportions of surfactant achieved higher IP, and, according to this test, T60B (containing 1.5 % w/v Tween® 60) was the most

Table 2 Induction period (IP), Protection Factor (PF) and apparent pH values for cupuassu fat (CF) and emulsions containing 80 % v/v oil phase and adding 0.5 or 1.5 % w/v Tween® 60, Tween® 80 or Tween® 85

Sample	IP/OSI (h)	PF at 120 °C (dimensionless)	pH
CB*	9–15	**	–
CF	44.58	**	4.02[a]
C80	31.56	1.00	***
T60A	19.05	0.60	4.63[b]
T60B	37.65	1.19	4.59[b]
T80A	9.84	0.31	4.70[c]
T80B	23.70	0.75	4.73 [cd]
T85A	13.31	0.42	4.77[d]
T85B	22.41	0.71	4.96[e]

Significant differences between pH values are indicated by lowercase letters between the types of emulsion according to the Tukey test ($p \leq 0.05$)

* CB Cocoa Butter (Metrohm 2013)

** PF was not considered

*** was not possible to measure the pH

stable emulsion, reaching 37.65 h of IP. This was the only emulsion to reach a PF higher than 1, indicating a good relationship between stability and antioxidant efficiency [19, 20], and this result is also in agreement with the data obtained in thermal stress and centrifuge tests, confirming its greater stability among all of the emulsions analysed. On the other hand, C80 presented an IP of 31.56 h, being resistant to oxidation even without co-adjuvants. The emulsion that was less resistant to oxidation and had a lower antioxidant efficiency was T80A, presenting 0.31 PF, and is therefore not an efficient surfactant to achieve high efficiency against oxidation [19].

Under the same Rancimat analysis conditions, cocoa butter (very similar to cupuassu fat), presented only 9–15 h of IP [21], being more susceptible to oxidation than cupuassu fat, which has higher resilience to oxidation due to the small amount of polyunsaturated fatty acids (2.6 %), since vegetable oils and fats with high unsaturation degree are more susceptible to lipid oxidation. In addition, contributing to this, its composition of saturated (53–58 %) and unsaturated (39–45 %) fatty acids is well balanced [1–3].

Through analysis of the apparent pH (Table 2) it can be seen that surfactants can also contribute to changes in the apparent pH of emulsions, which showed increases (changes from 0.57 to 0.94) when compared to cupuassu fat; also, some statistically significant differences were observed between all values according to the Tukey range test. This monitoring of pH provides information regarding the chemical stability of the formulation, i.e. a decrease of
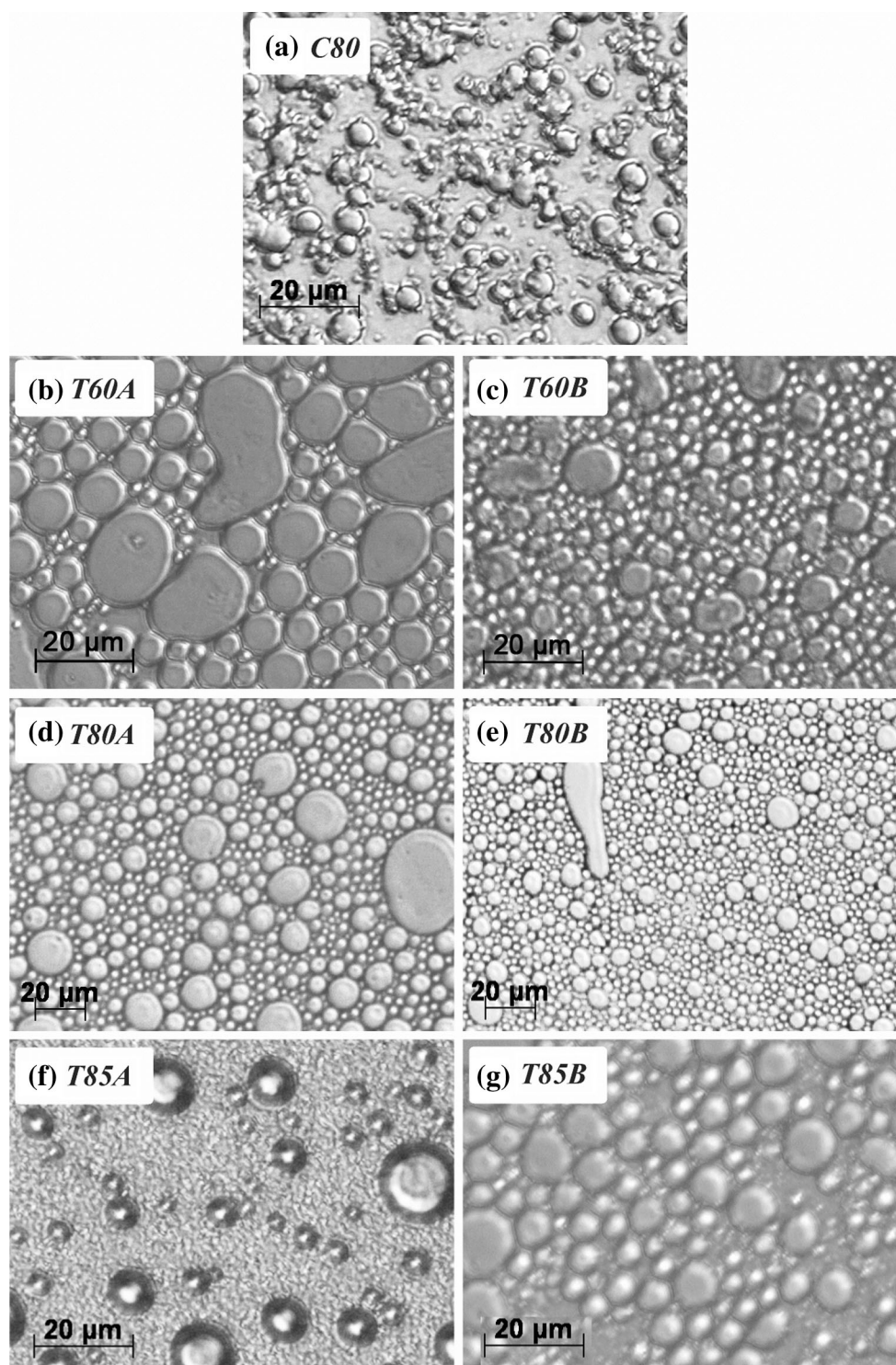
Fig. 3 Micrographs of emulsions produced with cupuassu fat containing 80 % (v/v) oil phase, without surfactant added (C80) (**a**), and with 0.5 % (left images) or 1.5 % (w/v) (right images) Tween® 60 (**b**, **c**), Tween® 80 (**d**, **e**) or Tween® 85 (**f**, **g**). Approximation of 640×; reference bar represent 20 µm

these values may be related to oxidation of the oily phase causing the formation of oxidised triglycerides or chain triglyceride hydrolysis, expressed by the formation of free fatty acids [48].

Despite differences in the values of apparent pH between surfactants, the most different pH value was obtained from the T85A emulsion, which was the least stable and showed a fluid appearance, as explained before;

Table 3 Droplet Size Distribution (DSD) for emulsions with 80 % (v/v) dispersed phase prepared at 43 ± 2 °C, stirring speed of 9000 rpm without surfactant addition (C80), and with 0.5 % or 1.5 % (w/v) Tween® 60, Tween® 80 or Tween® 85

Diameter range (μm)			Treatment				
	C80	T60A	T60B	T80A	T80B	T85A	T85B
0.0–4.9	76.20 ± 11.95[a]	47.20 ± 13.81[bc]	71.80 ± 13.37[ac]	67.00 ± 7.28[ac]	92.80 ± 3.35[a]	31.20 ± 22.55[b]	30.40 ± 16.01[b]
5.0–9.9	23.80 ± 11.95[bd]	47.00 ± 11.77[acd]	27.80 ± 13.59[bcd]	31.60 ± 7.83[cd]	6.80 ± 3.11[b]	48.20 ± 19.01[ac]	60.60 ± 10.36[a]
10.0–14.9	0.00 ± 0.00[b]	5.20 ± 5.36[b]	0.40 ± 0.55[b]	1.40 ± 0.89[b]	0.40 ± 0.55[b]	18.20 ± 8.23[a]	7.60 ± 9.07[b]
>15.0	0.00 ± 0.00[a]	0.60 ± 1.34[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	2.40 ± 1.52[a]	1.40 ± 1.95[a]

Results are expressed as percentage of the number of droplets and their respective standard deviation; significant differences are indicated by lowercase letters between the types of emulsion according to the Tukey test ($p \leq 0.05$)

the pH values for the samples containing Tween® 60 were similar ($p \leq 0.05$). In addition, it may be inferred that there was an interaction between the components of the emulsions, but these pH changes were not sufficient to harm the emulsions, as they continued to show acidic character (as fat) with values between 4.59 and 4.96.

Microscopy and Droplet Size Distribution

Micrographs of emulsions produced with cupuassu fat are shown in Fig. 3; Table 3 presents the droplet size distributions (DSD). The emulsions with a higher proportion of surfactant (Figs. 3c, e and g) yielded smaller droplets than the other emulsions (Figs. 3b, d and f). The control sample C80 (Fig. 3a) produced small droplets; however, they were mostly non-uniform due to the tendency of cupuassu fat to crystallise shortly after reaching temperatures around 25 °C [6, 9], causing the emulsion to coalesce and crystallise, thus reaching a solid state.

As seen in Table 3, there was a discrepancy in DSD in all treatments and significant differences between the ranges of droplet diameter were detected by the Tukey test ($p \leq 0.05$). Samples containing Tween® 80 (Figs. 3d and e) produced more uniform droplets, and T80B showed DSD over 90 % of droplets ranging in size from 1.1 to 4.9 μm (Fig. 3e and Table 3). It was found that, in this DSD range, the samples with the biggest percentage of DSD (C80 and T80B) showed no significant statistical differences, whereas all the other emulsions showed some differences.

The highest amount of droplets was measured in sizes ranging between 0.0 and 9.9 μm. Hence, samples with smaller droplet sizes were those containing Tween® 60 and Tween® 80, and these emulsions yielded greater stability, because in fact, a decrease in droplet size causes an increase in viscosity and emulsion stability [24, 49]. Although the control sample C80 (Fig. 3a) showed approximately 70 % of droplets ranging between 1.0 and 4.9 μm, as explained previously, these droplets were non-uniform with a rapid crystallisation, causing deformity.

It is noteworthy that the emulsions with Tween® 85 (Fig. 3f and g; Table 3) produced almost 70 % of the DSD ranging from 5.0 to over 15.0 μm, and beyond that, another image analysis (data not shown) revealed that it was the only emulsions presenting droplets > 20.0 μm. It is well known that the minimum size of stable droplets that can be produced during homogenisation is governed by the type and concentration of emulsifier [27], thus, with increasing the amount of surfactant, and applying a lower temperature, emulsions with Tween® 85 could achieve smaller droplets [13, 37, 38, 40] presenting higher stability. This emulsifier, which has a large lipophilic moiety [42] was not efficient enough to maintain a stable emulsion, thus producing larger droplets, possibly due to breakage of the thin film between colliding droplets (coalescence) or sharing of the adsorbed layer between two droplets (bridging flocculation) [23], and also because of the possible transitional inversion [13, 37], once the assays were performed at approximately 43 °C. Furthermore, once the emulsion interfacial area increases above a certain level, there may be insufficient emulsifier present to completely cover the surface of any newly formed droplets [27], thereby facilitating the process of coalescence of the emulsion.

Assuming that the surfactant molecules spontaneously arrange themselves into organised molecular assemblies (known as micelles), there is a threshold surfactant concentration required for the self-aggregation process, called the critical micellar concentration (CMC). Polysorbates form micelles at relatively low concentration levels, by way of example, the CMC for Tween® 60 in water is 0.021 mM, while for Tween® 80, is 0.010 mM, and for Tween® 85 is 0.00029 mM [36]. This is the concentration at which the polysorbate can form a monolayer. The concentration of emulsifier above the CMC governs its performance [39],thus, the three surfactants tested were well above the CMC. Besides that, for a fixed concentration of oil, water, and emulsifier, there is a maximum interfacial area that can be completely covered by an emulsifier [27]. Notwithstanding, as explained by Cottell & van Peij [39], under high temperature conditions the polysorbates form
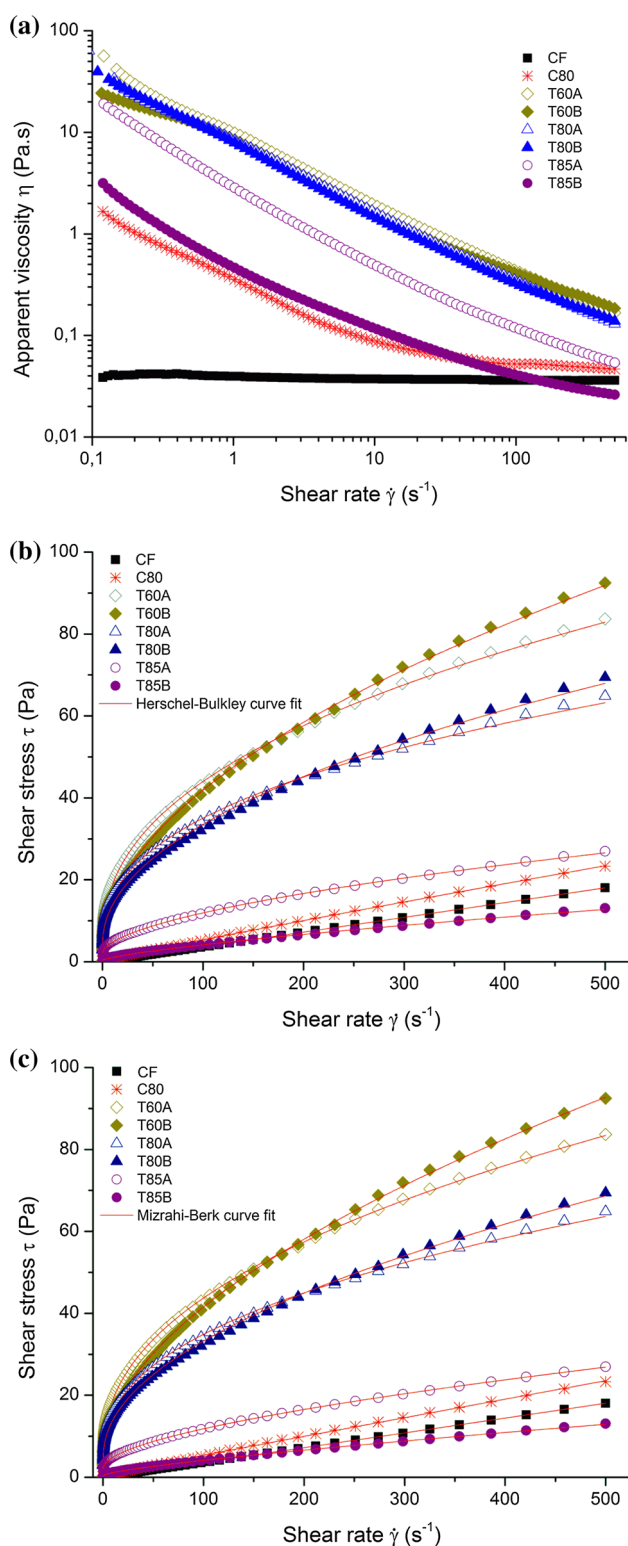
Fig. 4 Viscosity curves (**a**) representing shear rate and the dependence of apparent viscosity, and flow curves fitted by Herschel-Bulkley (**b**) and Mizrahi-Berk (**c**) models, representing shear rate and shear stress dependency, recorded at 45 °C in cupuassu fat (CF), emulsion without surfactant (C80), emulsions with 0.5 % (T60A, T80A and T85A) and 1.5 % (w/v) (T60B, T80B and T85B) surfactant

reverse micelles, swollen with water (w/o) with their ethoxylated chains directed inward and the non-polar fatty acid chains directed outward. As temperatures decrease, the polysorbate forms aqueous micelles that can hold oil (O/W).

Rheological Characterisation

Viscosity and flow curves are presented in Fig. 4. The viscosities of emulsions (Fig. 4a) were directly impacted by the surfactants added. Moreover, a decrease in the viscosity with shear rate increase was observed. The apparent viscosity was higher for those emulsions with a lower proportion of surfactant (0.5 % w/v) than the others produced with 1.5 % (w/v) surfactant. As shown in Fig. 4a, emulsions containing Tween® 60 and Tween® 80 showed similar results, exhibiting little differences between their viscosities and presenting the highest apparent viscosity for almost the entire range of shear rates tested, showing that is not necessary to add large amounts of these surfactants to increase viscosity properties, whereas the emulsions without surfactant (C80) and one containing 1.5 % (w/v) Tween® 85 (T85B) had lower apparent viscosity. Figure 4a also shows variation in the apparent viscosity of emulsions compared with the cupuassu fat (CF) viscosity, which does not really change, regardless of the applied shear rate.

The rheological behaviour of emulsions is strongly influenced by the droplet size, and faster stirring produces smaller and more uniform droplets, resulting in more viscous and stable emulsions [13]. Therefore, this increase in viscosity at low shear rates is related to the emulsions droplet concentration caused by the surfactant, leading to the appearance of strong non-Newtonian effects and the apparent viscosity dependence of the shear rate. This is also explained by the formation of an elastic interfacial layer at the drop surface; this elastic cover radically changes the boundary conditions between the two fluids and prevents the deformation of liquid inside the drops [50].

In accordance with rheograms (Fig. 4b and c), none of the emulsions exhibited a linear relationship between shear stress and shear rate, thus demonstrating non-Newtonian behavior. In order to represent the flow behaviour of these fluids, the Herschel-Bulkley (H) and Mizrahi-Berki (M) rheological models were used. Values of K (consistency coefficient—mPa s^n) and n (flow behaviour index—dimensionless) are presented in Table 4. For the two rheological models, the determination coefficient (R^2) values were higher than 0.99, and the Chi square (χ^2) values lower than 1.54; these results show a good fit for both H and M models (Table 4). All the samples exhibited shear-thinning behaviour, since the values of n were lower than 1, the

Table 4 Rheological parameters fitted to the Herschel-Bulkley and Mizrahi-Berk models for cupuassu fat (CF) and emulsions with 80 % (v/v) dispersed phase prepared at 43 ± 2 °C, stirring speed of 9000 rpm, without surfactant (C80) and using 0.5 % or 1.5 % (w/v) Tween® 60, Tween® 80 or Tween® 85 and analysed at 45 °C

Model	Treatment	Parameters				
		τ_{0H} (mPa)	K_H (mPa.s^n)	n_H	R^2	χ^2
Herschel-Bulkley	CF	0.007 ± 0.001	0.036 ± 0.000	0.999 ± 0.001	0.99968	0.0001
	C80	0.255 ± 0.009	0.067 ± 0.001	0.940 ± 0.003	0.9997	0.004
	T60A	3.502 ± 0.205	5.895 ± 0.146	0.418 ± 0.004	0.9976	0.527
	T60B	4.010 ± 0.270	3.417 ± 0.146	0.522 ± 0.007	0.9940	1.540
	T80A	2.429 ± 0.204	5.503 ± 0.156	0.387 ± 0.005	0.9967	0.425
	T80B	4.212 ± 0.215	3.186 ± 0.131	0.482 ± 0.007	0.9940	0.821
	T85A	2.114 ± 0.048	0.721 ± 0.023	0.567 ± 0.005	0.9970	0.058
	T85B	0.380 ± 0.014	0.125 ± 0.004	0.740 ± 0.005	0.9982	0.008
		K_{0M} (mPa)	K_M (mPa.s^n)	n_M	R^2	χ^2
Mizrahi-Berk	CF	0.026 ± 0.002	0.184 ± 0.001	0.504 ± 0.000	0.999999	0.00005
	C80	0.345 ± 0.014	0.184 ± 0.004	0.515 ± 0.003	0.9994	0.008
	T60A	1.401 ± 0.052	1.686 ± 0.043	0.245 ± 0.003	0.9984	0.346
	T60B	1.621 ± 0.067	1.131 ± 0.047	0.315 ± 0.006	0.9958	1.071
	T80A	1.141 ± 0.061	1.696 ± 0.053	0.224 ± 0.004	0.9976	0.303
	T80B	1.741 ± 0.050	1.002 ± 0.037	0.302 ± 0.005	0.9963	0.506
	T85A	1.300 ± 0.017	0.388 ± 0.011	0.371 ± 0.004	0.9980	0.039
	T85B	0.511 ± 0.008	0.199 ± 0.003	0.441 ± 0.002	0.9994	0.002

R^2 coefficient of determination, χ^2 Chi square

most common behavior found in emulsions [18]. In all these cases, the oil phase structuration is achieved by the creation of a fat crystalline network inside which water droplets are entrapped [15], causing the shear-thinning behaviour, once the droplets are deformed in their shape but they are entrapped in this network [51]. In addition, at higher fat concentrations the droplets tend to be packed fairly densely into the system [24]. Likewise, similar behavior has been reported for aqueous systems using a mixture of cationic and anionic surfactants [29] and for multilamellar vesicles (MLVs) [51, 52].

Cupuassu fat (CF) sample and control emulsion (C80) showed almost Newtonian behaviour ($n_H \geq 0.9$) according to the H model, assuming that oils are usually Newtonian, but at very high shear rates, in the shear stress x shear rate diagram, there may be a curvature toward the axis of the shear rate, referring to the as pseudoplasticity [46]. On the other hand, when the data were fitted to the Ostwald-de Waele model, Lannes et al. [46] stated that clearly CF presents non-Newtonian behavior ($n = 0.83$).

Rheological data confirm that the T60A emulsion was more uniform, had the best performance and greater consistency for the H model ($K_H = 5.895 \pm 0.146$) than the other samples, while the M model showed both T60A ($K_M = 1.686 \pm 0.043$) and T80A ($K_M = 1.696 \pm 0.053$) emulsions to be equally more consistent. Furthermore, T85A and T85B emulsions had the worst performance

($K_H = 0.721$ and 0.125 and $K_M = 0.388$ and 0.199, respectively), given their low stability, and the models show that they, along with the emulsion without surfactant (C80), were less consistent ($K_H = 0.067$ and $K_M = 0.184$).

Dynamic Mechanical Analysis

Oscillatory tests were performed in order to determine the effect on the mechanical response of the analysed emulsions. The oscillatory tests are sensitive to changes in chemical composition and physical structure of the samples; the analysis consisted of testing the samples in a nondestructive way. The continuous shear disrupts the emulsion and thus significant differences between formulations are not displayed by the static tests; however, using the oscillatory technique, the sample maintains its structure, and consistency can be distinguished between the emulsions. The results showed that emulsions presented strong gel characteristics and those with lower surfactant (0.5 % w/v) were more significant in this regard.

As shown in Fig. 5, the mechanical spectra of emulsions (obtained by the viscoelastic properties analysis) show that samples containing 0.5 % (w/v) (Fig. 5a) Tween® 60 and Tween® 80 exhibit the typical behaviour of rigid gels with storage modulus (G') values higher than the loss modulus (G'') throughout the entire frequency range (0.01–10.00 Hz). Also, both moduli should be almost
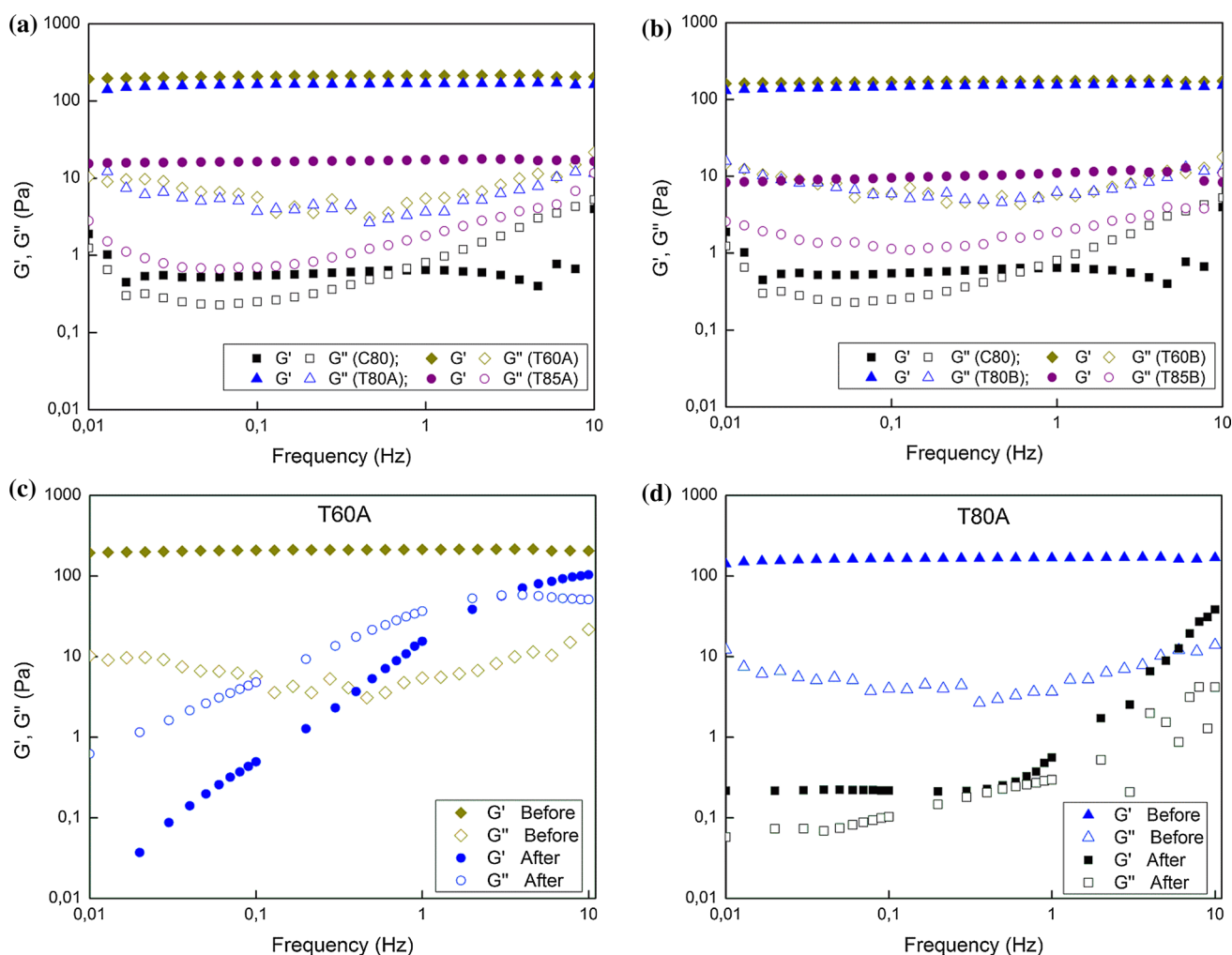
Fig. 5 Frequency sweeps showing the frequency dependence of G' (*full symbols*) and G'' (*empty symbols*) at 45 °C (τ = 1.0 Pa), to cupuassu fat emulsions with 0.5 % (**a**) and 1.5 % (w/v) (**b**) Tween[®] 60, Tween[®] 80 and Tween[®] 85, compared to the emulsion without surfactant (C80), and frequency sweeps performed before and after the flow curve to emulsions with 0.5 % (w/v) Tween[®] 60 (**c**) and Tween[®] 80 (**d**)

parallel throughout the observed frequencies, with a slight increase of G'' at high frequencies (ω >1.0 Hz), featuring viscoelastic solid behaviour with a gel-like structure [18].

The mechanical properties of the evaluated emulsions showed differences that are likely related to the nature of the emulsifier added. It was found that the two tested concentrations of Tween[®] 60 and Tween[®] 80 had little influence on the G'' evolution at the tested frequency. On the other hand, the emulsion without surfactant (C80) exhibited a frequency dependence with higher values of G'' at frequencies >1.0 Hz, showing gel behaviour at low frequencies (ω < 1.0 Hz). Compared to emulsions prepared with 0.5 % (w/v) surfactant (Fig. 5a), values of G' and G'' over the frequency did not change significantly with the increase of surfactant (Fig. 5b) for all samples.

Once verified that increasing surfactant implies little difference in G'' evolution, two samples containing 0.5 %

(w/v) surfactant (Tween[®] 60 and Tween[®] 80) were chosen to be subject for dynamic rheological analysis also after the flow curve (Fig. 5c, d), in order to evaluate the existence of structures, comparing with the frequency sweep performed before the flow curve. It was meaningful to observe that after being subjected to shear, the emulsion containing Tween[®] 60 (Fig. 5c), changed completely its behavior, since the loss modulus (G'') was higher than the storage modulus (G') at low frequencies (ω < 1.0 Hz), whereupon there was reversal of values and the storage modulus became higher (ω > 1.0 Hz), presenting the behavior of a concentrated solution and therefore, showing a clear tendency for more solid-like behaviour at higher frequencies. In contrast, the shear caused minor modifications in the sample containing Tween[®] 80 (Fig. 5d), and the gel behaviour has not been modified, because despite the reduction in G' and G'' values, the sample structure is still
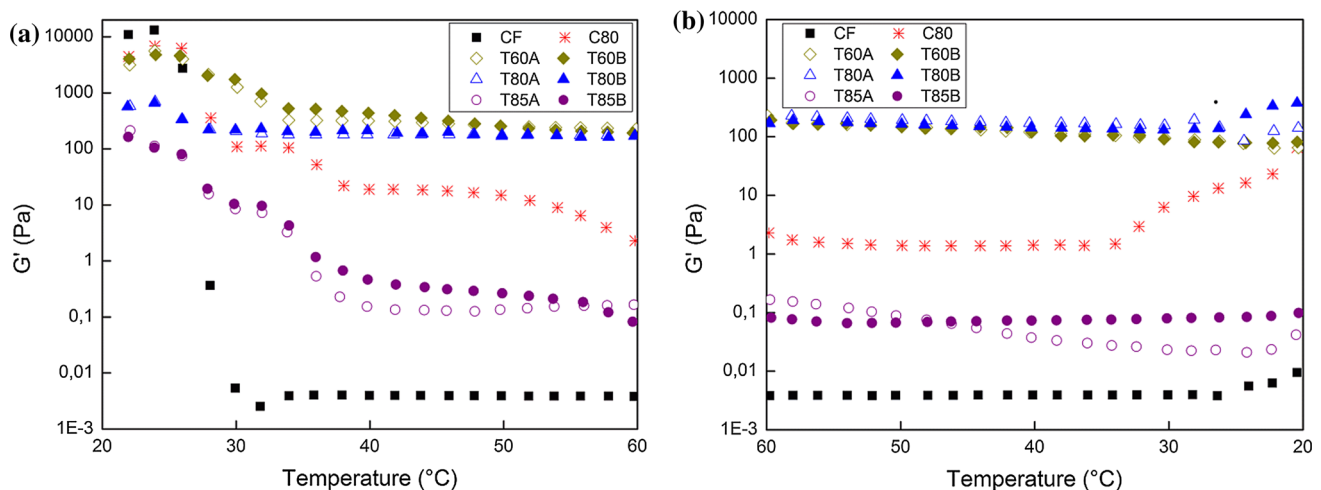
Fig. 6 Sweep temperature on heating (**a**) (20–60 °C) and cooling (**b**) (60–20 °C) cycle with rate of 2 °C/min, showing G' evolution in emulsions containing 0.5 % and 1.5 % (w/v) Tween® 60, Tween® 80 and Tween® 85, compared to cupuassu fat and the emulsion without surfactant

gel, since G' remained greater than G''. In order to understand how the surfactant (type/amount) in cupuassu fat emulsions is affected by temperature, the viscoelastic properties were studied during the application of a temperature ramp ranging from 20 to 60 °C, emphasising the temperature dependence of G', as shown in Fig. 6a and b.

Upon heating (Fig. 6a), all samples show mechanical transitions (a sigmoidal reduction in G') from 35 °C, related to the melting temperature of cupuassu fat [4, 6, 9], revealing lower network resistance formed by the structured emulsion, and suggesting structural changes in the network of crystals of the samples [15]. During cooling (Fig. 6b), samples did not return to their original G' values after reaching 20 °C. Nevertheless, emulsions with Tween® 60 and Tween® 80 (T60A, T80A, T60B, T80B) showed less fluctuation in the G' values, confirming their higher stability at high temperatures, whereas emulsions containing Tween® 85 (T85A, T85B) presented smaller G' values, probably due to the coalescence and phase inversion that occurred after the application of high temperatures. Considering that emulsions are thermodynamically unstable, given enough time, droplets become larger, which leads to the macroscopic breakdown of less stable emulsions [47].

Conclusion

In the present research, a comparative investigation was made on rheological and microstructural properties of emulsions made with cupuassu fat plus the addition of polyoxyethylene sorbitan surfactants. The experimental results showed that the emulsions stability was increased, being positively influenced by the concentration of the surfactants (0.5 and 1.5 % w/v), and that those containing Tween® 60 and Tween® 80 presented the best results in

overall tests. The possibility to obtain almost the same emulsion characteristics using less surfactant is a positive effect. On the other hand, overall, the emulsions without surfactant or with Tween® 85 reached unsatisfactory results. For the first time, the oxidative stability of cupuassu fat was established by Rancimat. Furthermore, the rheological behaviour of cupuassu fat emulsions was appropriately described by the Herschel-Bulkley and Mizrahi-Berk models, presenting a non-Newtonian behaviour of pseudoplastic character; the oscillatory tests showed that all emulsions were strong gels. All of these data enable a better understanding of the structural basis of fat emulsion rheology and have implications for the design and production emulsions with fat in their composition; these are also important for designing pipes, pumps and equipment according to the viscosity and rheological properties of the product.

Compliance with Ethical Standards

Conflict of interest The authors have declared no conflict of interest.

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