

Article

Comparative Study of Physicochemical Properties of Nanoemulsions Fabricated with Natural and Synthetic Surfactants

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Abstract: This work aims to evaluate the effect of two natural (whey protein isolate, WPI, and soy lecithin) and a synthetic (Tween 20) emulsifier on physicochemical properties and physical stability of food grade nanoemulsions. Emulsions stabilized by these three surfactants and different sunflower oil contents (30% and 50% *w/w*), as the dispersed phase, were fabricated at two levels of homogenization pressure (500 and 1000 bar). Nanoemulsions were characterized for droplet size distribution, Zeta-potential, rheological properties, and physical stability. Dynamic light scattering showed that droplet size distributions and D_{50} values were strongly affected by the surfactant used and the oil content. WPI gave similar droplet diameters to Tween 20 and soy lecithin gave the larger diameters. The rheology of emulsions presented a Newtonian behavior, except for WPI-stabilized emulsions at 50% of oil, presenting a shear-thinning behavior. The physical stability of the emulsions depended on the surfactant used, with increasing order of stability as follows: soy lecithin < Tween 20 < WPI. From our results, we conclude that WPI is an effective natural replacement of synthetic surfactant (Tween 20) for the fabrication of food-grade nanoemulsions.

Keywords: O/W emulsions; surfactants; interfacial tension; droplet size; rheology; stability

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1. Introduction

In the last decades, progress in the design of delivery systems for drug solubilization/encapsulation has enabled important advances in the study of emulsion-based matrices for this purpose. Thus, different delivery systems have been designed to encapsulate, protect and/or deliver specific components (e.g., vitamins and nutraceuticals) at controlled rates to specific sites within the human body [1,2]. A wide variety of emulsion-based delivery systems have been developed for food and pharmaceutical applications, including nanoemulsions.

Nanoemulsions are colloidal dispersions used as drug vehicles constituted of safe grade excipients and are composed of particles within a nanometer range [3]. They are non-equilibrium systems with characteristics and properties that depend on their composition and fabrication method [4]. Particularly, oil-in-water (O/W) nanoemulsions consist of small oil droplets dispersed within an aqueous medium, with each oil droplet being surrounded by a protective coating of surfactant/emulsifier [3–6]. This kind of emulsions shows several advantages in comparison with conventional emulsions such as: (i) they have an improved physical stability to particle aggregation and gravitational separation, (ii) they can be fabricated in variety of formulations, (iii) they scatter light weakly and so

can be incorporated into optically transparent products, and (iv) they improve the bioavailability of hydrophobic substances [3,4,7–9].

Nanoemulsion formation is a critical point to understand the potential applications of these matrices in the food and pharmaceutical industry. For example, applications like encapsulation of lipophilic components, increased bioavailability and controlled delivery of lipophilic components, improved emulsion stability, texture modification and antimicrobial activity will be determined by the formulation conditions chosen to give with the type of nanoemulsion required for a particular use [10].

O/W nanoemulsions are composed by three main components: oil phase, aqueous phase, and surfactant. In relation to the oil phase, physicochemical characteristics of oils (e.g., interfacial tension, viscosity, and chemical stability) limit the type of homogenization method (i.e., high-energy and low-energy methods) that can be used to fabricate nanoemulsions [7,11,12]. Particularly, high-energy (pressure) homogenization is the technique more effective and more used in practice, which employs mechanical tools to induce disruptive forces to break up and blend the oily phase and oil droplets. In fact, recent studies continue to demonstrate the effectiveness of this technique for (nano)emulsion fabrication [13–22], even when using essential oils [23–26]. On the other hand, the aqueous phase, which typically consists of water, can contain other polar components (e.g., co-solvents, minerals, acids, and bases) where the type and concentration of these components determines the polarity, interfacial tension, rheology, pH, and ionic strength, to name just a few prominent examples that influence the formation, stability, and physicochemical properties of the nanoemulsion [10]. However, the most important factor for the proper design of a nanoemulsion is the correct selection of the emulsifier (or mix of them), because during fabrication of nanoemulsion is required that: (i) the emulsifier promotes a very low interfacial tension, which favors the production of small droplets when using high-energy fabrication methods, or facilitates the spontaneous formation of small droplets when applying low-energy approaches, (ii) the emulsifier concentration must be high enough to provide the amount of surfactant required to stabilize the new droplets formed, and (iii) the oil/surfactant interface must be flexible enough to induce the formation of nanoemulsion [4,7,10,27]. In addition, the kind of emulsifier used for the fabrication of nanoemulsions also impacts the stability of the emulsion to environmental stresses encountered in foods (e.g., elevated temperatures, pH, ionic strength, and long-term storage) [10,28].

Nowadays, food and process engineers and food technologists are addressing new trends to innovate in emulsion technology. Interest in this field continues to grow, resulting in a wealth of new information on the potential applications of nanoemulsions according to their physicochemical properties mainly determined by the kind of emulsifier used in their formulations. For example, synthetic emulsifiers (e.g., Tweens and Spans) are recognized for serving as good stabilizing agents; however, today consumers demand safe, natural, and healthy food products [29,30]. Under this scenario, natural emulsifiers (e.g., proteins and phospholipids) are good alternatives to synthetic emulsifiers, but study of the physicochemical properties of these matrices still requires investigation in order to prospect their future applications in diverse fields.

Based on the above, the objective of this work was to evaluate the effect of the type of emulsifiers in the development of nanoemulsions based on sunflower oil, comparing two natural (whey protein isolate and lecithin) and a synthetic (Tween 20) emulsifiers on physical properties (interfacial tension at the oil/water interface, oil droplet size, Zeta-potential, and rheology) and physical stability of these nanoemulsions.

2. Materials and Methods

2.1. Raw Materials

Sunflower oil (Natura™, Argentina) was purchased from a local supermarket. Three surfactants were used for the experiments: Tween 20 (Sigma-Aldrich, St. Louis, MO,

USA), whey protein isolate (WPI) (BIPRO™, Davisco Foods International Inc., Le Sueur, MN, USA) and l-alfa-soy lecithin (Acros Organics, USA). The hydrophilic-lipophilic balance (HLB) value for the three surfactants used was: Tween 20 (HLB: 16.7), whey protein isolate (HLB: 9.1) and soy lecithin (HLB: 4.5). Ultra-pure water (resistivity of 15 MΩ-cm) was used for the preparation of all samples.

2.2. Fabrication of Emulsions

Emulsions were prepared from a lipid and an aqueous phase, mixing lipid phase (sunflower oil) at 30% or 50% (*w/w*) with aqueous phase (emulsifier dispersion) at 70% or 50% (*w/w*). Emulsifier dispersions were prepared to reach a mass ratio 15:1 (dispersed phase:surfactant ratio) into sodium phosphate buffer 5 mN (pH 7.0). Emulsifier dispersions were subjected to continuous stirring for at least 1 h and at 25 °C. Then, they were kept overnight at 5 °C before emulsification.

Pre-emulsions (PE) were elaborated at room temperature by dispersing dropwise the lipid phase into the aqueous phase using a rotor-stator homogenizer (Kinematica Polyttron®, PT 2500 E, Switzerland) operated at 15,000 rpm for 3 min. The pH of the PE was adjusted to 7.0 if required. These PE were processed in a high-pressure homogenizer (Gea Niro Soavi, model Panda Plus, Italy) operating at 500 or 1000 bar and at 5 homogenization steps. All the experiments were performed in triplicate.

2.3. Measurements of Dynamic Interfacial Tension (DIT) between Surfactant Dispersions and Sunflower Oil

Changes in the interfacial tension between the lipid phase (sunflower oil) and the three surfactants were determined by using an optical tensiometer (Ramé-Hart Inc., model 250-F4, NJ, USA). Measurements of DIT were based on the pendant drop method. In this method, an axisymmetric drop (~9 µL) of surfactant dispersion was delivered and allowed to stand at the tip of a steel needle inside a quartz cell with 30 mL of sunflower oil for one hour at 25 °C to achieve surfactant adsorption at the oil-water interface. Two concentrations of surfactant (2.86% and 6.66% *w/w*) were analyzed in order to keep the 15:1 mass ratio between dispersed phase:surfactant employed in the emulsion fabrication.

The CCD camera of the optical tensiometer captured drop images at different time intervals for one hour. The DIT was calculated by analyzing the image profile of the drops stabilized by the surfactant dispersions by means of image analysis using the DROPimage Advanced software (Ramé-Hart Inc., Succasunna, NJ, USA) and then by fitting the Laplace equation to the drop shape. To validate the methodology, it was experimentally corroborated that the interfacial tension of the sunflower oil/pure water system (26.6 ± 0.5 mN/m) was almost the same as previously reported (26.45 ± 0.46 mN/m) for identical conditions [31].

Dynamic interfacial tension curves were fitted (CurveExpert Professional, version 2.6, Hyams Development, Madison, AL, USA) to an empirical model [32] to quantify the rate of interfacial tension decrease.

$$\tau_t = \tau_0 + k \ln(t) \quad (1)$$

where τ_t is the interfacial tension at time t (mN/m), τ_0 is the interfacial tension at time = 0 (mN/m), and k is the interfacial tension decay rate (mN/m s).

2.4. Measurement of Droplet Size Distributions of the Emulsions

Oil droplet size distribution of the emulsions was evaluated by using a dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). Emulsion samples were diluted with phosphate buffer at a ratio 1:500 (*v/v*) before measurements in order to avoid multiple scattering. The results were calculated by the instrument software. Cumulative frequency curves were determined from the droplet size distribution curves. These curves were fitted to empirical models (CurveExpert Professional,

version 2.6, Hyams Development, Madison, AL, USA) and droplet diameter at 10% (D_{10}), 50% (D_{50}), and 90% (D_{90}) of the cumulative frequency distribution were obtained.

The dispersity of the oil droplets generated after emulsification was estimated using the particle size dispersal coefficient, DC [33,34]. Lower DC values imply more homogeneous droplet distributions.

$$DC = \frac{D_{90} - D_{10}}{D_{50}} \quad (2)$$

2.5. Zeta-Potential Measurements

Emulsions samples were diluted with milli-Q water prior to analysis at a ratio of 1:100 (v/v), by placing them in plastic zeta cells (DTS1061, Malvern, UK). Afterwards, Zeta-potential was evaluated at 25 °C (Zetasizer Nano-ZS, Malvern Instruments, Worcester-shire, UK) to determine the surface charge of particles in dispersion.

2.6. Rheological Characterization of the Emulsions

The flow behavior of the emulsions was evaluated in a controlled shear rate rheometer (Anton Paar, ReolabQC, Austria) with a concentric cylinder geometry conforming to DIN 53018. Emulsion samples were carefully poured into the rheometer cup and allowed to stand 2 min before shearing. The test temperature was set at 25 °C using a Peltier temperature device (Peltier RheolabQC plus, C-PTD180/AIR/QC). The rheological characterization of emulsions was performed using a flow curve test, where the shear rate was increased linearly from 1 to 200 s^{-1} .

For the range of shear rates studied, the experimental flow curves obtained of the emulsions were adjusted to the Power Law model described as:

$$\sigma = K \times \dot{\gamma}^n \quad (3)$$

where σ is the shear stress (Pa), K is the consistency coefficient ($Pa s^n$), $\dot{\gamma}$ is the shear rate (s^{-1}) and n is the flow behavior index (dimensionless). For a Newtonian emulsion $n = 1$ and for an emulsion which exhibits shear-thinning behavior $n < 1$. To compare the different rheological behaviors of the emulsions, the apparent viscosity at a shear rate of 60 s^{-1} was evaluated because the human perception of thickness is correlated to the apparent viscosity at this shear rate [35].

2.7. Physical Stability of Emulsions

The physical stability of the emulsions was evaluated by static multiple light scattering using a vertical scan analyzer (TurbiScan MA2000, Formulation, Toulouse, France). The backscattering (BS) and transmission intensities of an incident near-infrared light ($\lambda = 880$ nm) were both measured automatically every 40 μm along the cylindrical glass cell. The BS intensity variations as function of the sample height (70 mm) provided a qualitative assessment of the distribution and movements evolution of the oil droplets during destabilization. Samples of emulsion (~7.5 mL) were placed into cylindrical glass tubes and stored at 5 °C. The BS profile was then measured at room temperature (25 °C) during a storage time of seven days.

The Turbiscan Stability Index (TSI) is a statistical parameter used to estimate the dispersion stability. This index considers the sum of all destabilization processes occurring in the emulsion samples. Thus, the stability results are presented as TSI, which were calculated by the following expression [32,36,37]:

$$TSI = \sum_{t=1} \frac{\sum_{h=0}^{h=H} |BS_t(h) - BS_{t-1}(h)|}{H} \quad (4)$$

where BS is the backscatter intensity, t is the scanning time, h is the height of the measurement per 40 μm , and H is the height of the sample in the measuring cell.

2.8. Statistical Analysis of Data

Analysis of variance (ANOVA) tests, at a confidence level of 95%, were used to analyze the data using the Statgraphics Centurion 18 software (Statgraphics Technologies, Inc., The Plains, VA, USA). Differences between samples were evaluated using multiple range test, by means of the least significant differences (LSD) multiple comparison method.

3. Results and Discussion

Nanoemulsions were successfully fabricated using a food grade synthetic (Tween 20) and two natural (WPI and soy lecithin) emulsifiers at two levels of dispersed phase (sunflower oil) 30% and 50% by weight. Different physicochemical properties of these nanoemulsions were determined, in order to anticipate their future applications in diverse fields.

3.1. Dynamic Interfacial Tension (DIT) at the Oil/Water Interface

It is widely recognized that the capacity of amphiphilic molecules to decrease the interfacial tension between the dispersed and continuous phase is critical in the formation of droplets and the stability of emulsions [4,7,10]. In this sense, we measured the DIT for the three surfactants used in this study. The concentrations employed in these measurements were the same as used for the fabrication of nanoemulsions, keeping the mass ratio at 15:1 (dispersed phase:surfactant). To fabricate stable emulsions, an excess of emulsifier in the formulation is necessary, because there is an equilibrium between emulsifier at the droplet surface and that in the continuous phase that must be achieved for adequate kinetic stability [38].

The rate at which an emulsifier adsorbs to an interface is one of the important factors to consider during emulsion formation. For the three emulsifiers used in this research, the rate of decrease in DIT was faster at early stages of adsorption, being more evident for soy lecithin (Figure 1). The faster decrease in DIT at early stages is due to diffusion of emulsifier molecules to the oil/water interface. Then, the rate of decrease in DIT diminished because of the saturation of the oil/water interface. For Tween 20 it was observed that the interfacial tension reached relatively constant values after 1800 s, which could indicate the complete saturation of the oil/water interface by emulsifier molecules [37,39], reaching an equilibrium. This behavior (equilibrium) was not evident for WPI and soy lecithin, needing longer experimental times to reach equilibrium values. It is known that proteins unfold and rearrange their secondary and tertiary structure once adsorbed at liquid interfaces [40–42], whereas phospholipids are able to form self-assembling supramolecular structures (e.g., lamellar and/or hexagonal) at the interface [43,44]. The molecular weight influences the kinetics of adsorption of amphiphilic molecules at the oil/water interface. It is recognized that low molecular weight surfactants can decrease the interfacial tension in a greater extension than high molecular weight surfactants, but high molecular weight surfactants are more effective in the formation of a viscoelastic film surrounded oil droplets, which favors the stabilization of emulsions [32,45,46]. It was observed that Tween 20 and soy lecithin showed lower DIT values than WPI (Figure 1), due to the lower molecular weight of Tween 20 (1227 Da) and soy lecithin (750 Da) in comparison with the average molecular weight of the main proteins in WPI (18.4 kDa) [34].

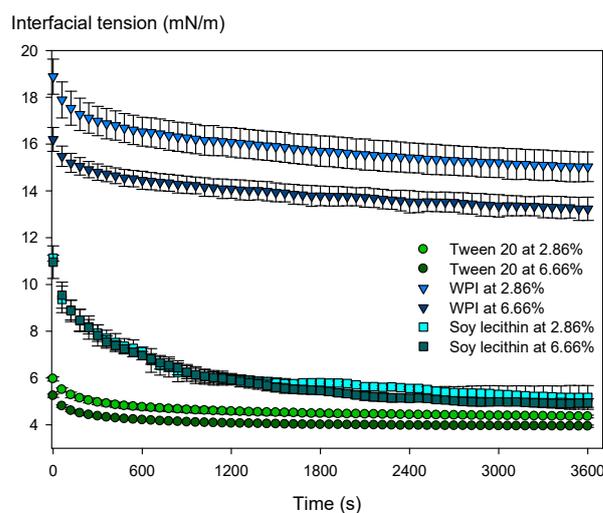


Figure 1. Dynamic interfacial tension at oil/water interface for the three surfactants studied. Error bars represent one standard deviation.

The emulsifier concentration also influenced DIT values since the interfacial tension values decreased as the emulsifier concentration increased (Figure 1). The decrease in interfacial tension as increasing emulsifier concentration can be related to a faster emulsifier adsorption to the oil/water interface, due to the higher number of surfactant molecules present in the aqueous phase. However, analyzing the k values after one hour of droplet stabilization (Table 1), it is possible to see that only the type of emulsifier had a significant effect ($p < 0.05$) on these values. However, it is clear from Figure 1, that the interfacial tension after one hour of droplet stabilization was lower for the systems with the higher amount of surfactant. Literature values for the interfacial tension between sunflower oil and pure water are about 25–26 mN/m [31,32,36], which decreased to approximately 5 mN/m in the presence of Tween-20, a small nonionic surfactant [47], results in agreement with those found in this work (Table 1). On the other hand, Bai et al. [34] found an interfacial tension value of 9 mN/m between WPI at 1% (w/w) and corn oil, whereas Gomes et al. [32] found a value of 20 mN/m for the same concentration of WPI but using sunflower oil, our results are in the middle of these two previous works. Arancibia et al. [37] reported higher values (8–10 mN/m) of interfacial tension for soy lecithin and avocado oil than those reported here, probably because of different processes of isolation and purification applied for different commercial brands of soy lecithin.

Table 1. Fitting parameters of the dynamic interfacial tension curves.¹

Surfactant concentration (% by Weight)	Tween 20		Whey Protein isolate		Soy Lecithin	
	τ_0 (mN/m)	k (mN/m s)	τ_0 (mN/m)	k (mN/m s)	τ_0 (mN/m)	k (mN/m s)
2.86	6.47 ± 0.17 ^{aB}	-0.263 ± 0.011 ^{cA}	21.20 ± 0.73 ^{cB}	-0.739 ± 0.019 ^{bA}	13.81 ± 0.18 ^{bB}	-1.056 ± 0.007 ^{aA}
6.66	5.49 ± 0.03 ^{aA}	-0.195 ± 0.002 ^{cA}	18.08 ± 0.24 ^{cA}	-0.576 ± 0.026 ^{bA}	14.55 ± 1.01 ^{bA}	-1.204 ± 0.129 ^{aA}

¹ Different lower-case letters indicate significant differences ($p < 0.05$) between surfactants. Different capital letters indicate significant differences ($p < 0.05$) between surfactant concentration.

3.2. Effect of the Formulation and Processing Conditions on Droplet Size Distribution of Emulsions

Cumulative frequency curves were calculated from the droplet size distribution curves and relevant droplet diameters (D_{10} , D_{50} , D_{90}) were obtained at different cut-off on the cumulative frequency curve (Table 2). For D_{50} and D_{90} values, emulsions fabricated with soy lecithin gave statistically higher values ($p < 0.05$) than Tween 20 and WPI; but contrary results were found for D_{10} , where soy lecithin presented the lower values compared with Tween 20 and WPI. In general, small molecule surfactants can lead to smaller droplets sizes, which it has been attributed to faster adsorption rates [45]. However, WPI gave similar droplet diameters to Tween 20 and soy lecithin gave the larger diameters, although their capacity to diminish the interfacial tension was similar to Tween 20 (Figure 1; Table 1). Perhaps soy lecithin is not as effective as WPI to avoid coalescence during the homogenization process to fabricate the nanoemulsions, and larger droplet diameters are obtained under the same operational conditions. Using Tween 20, Silva et al. [45] obtained hydrodynamic droplet diameters between 171 and 341 nm, with homogenization pressures between 690 and 1380 bar and using from 10 to 30 homogenization cycles. Larger droplet diameters ($D_{4,3}$) for emulsions fabricated with soy lecithin in comparison with Tween 20 were found by Ferreira et al. [47], in agreement with our results. Flores-Andrade et al. [39] found smaller droplet sizes (<160 nm) for emulsions fabricated with WPC and soy lecithin, but the emulsification pressure was about 1500 bar.

Table 2. Droplet diameter and dispersal coefficient obtained from the cumulative frequency distributions of oil droplet size for the three surfactants studied. ¹

Surfactant	Oil Content (% w/w)	Emulsification Pressure (bar)	D_{10} (nm)	D_{50} (nm)	D_{90} (nm)	Dispersal Coefficient (Dimensionless)	Distribution Shape
Tween 20	30	500	141 ± 3 ^{bA}	251 ± 8 ^{aA}	399 ± 15 ^{aA}	1.03 ± 0.02 ^{aA}	Monomodal
	30	1000	145 ± 0 ^{bA}	237 ± 2 ^{aA}	349 ± 7 ^{aA}	0.86 ± 0.02 ^{aA}	Monomodal
	50	500	173 ± 5 ^{bB}	288 ± 4 ^{aA}	427 ± 4 ^{aA}	0.88 ± 0.03 ^{aA}	Monomodal
	50	1000	202 ± 8 ^{bB}	332 ± 16 ^{aB}	503 ± 52 ^{aA}	0.90 ± 0.09 ^{aA}	Monomodal
Whey protein isolate	30	500	136 ± 10 ^{bA}	262 ± 24 ^{aA}	508 ± 83 ^{aA}	1.44 ± 0.42 ^{aA}	Bimodal
	30	1000	115 ± 6 ^{bA}	194 ± 7 ^{aA}	316 ± 20 ^{aA}	1.04 ± 0.17 ^{aA}	Monomodal
	50	500	173 ± 10 ^{bB}	302 ± 6 ^{aA}	537 ± 29 ^{aA}	1.20 ± 0.12 ^{aA}	Bimodal
	50	1000	131 ± 3 ^{bB}	214 ± 6 ^{aB}	326 ± 26 ^{aA}	0.91 ± 0.10 ^{aA}	Monomodal
Soy lecithin	30	500	121 ± 19 ^{aA}	344 ± 19 ^{bA}	1016 ± 340 ^{bA}	2.65 ± 1.23 ^{bA}	Bimodal
	30	1000	67 ± 3 ^{aA}	146 ± 11 ^{bA}	935 ± 655 ^{bA}	5.91 ± 4.38 ^{bA}	Bimodal
	50	500	120 ± 16 ^{aB}	310 ± 1 ^{bA}	780 ± 171 ^{bA}	2.13 ± 0.50 ^{bA}	Bimodal
	50	1000	351 ± 15 ^{aB}	615 ± 46 ^{bB}	1145 ± 154 ^{bA}	1.29 ± 0.19 ^{bA}	Bimodal

¹ Different lower-case letters indicate significant differences ($p < 0.05$) between surfactants. Different capital letters indicate significant differences ($p < 0.05$) between process conditions.

In terms of the operational conditions (oil content and homogenization pressure), D_{10} values showed statistical differences ($p < 0.05$) for the oil content incorporated, independent of the homogenization pressure. Emulsions with 30% of oil content presented lower D_{10} values than emulsions formulated at 50% of oil content. For D_{50} , the combination of 50% of oil content and 1000 bar of homogenization pressure resulted in statistically higher values than the other three combinations of oil content and homogenization pressure. No effect of oil content or homogenization pressure was found for D_{90} values. Although higher pressures induce higher shear forces during homogenization, droplets diameter (D_{50}) were smaller only when 30% of dispersed phase was used. At 50% of oil phase, there

is probably not enough surfactant present in the continuous phase to generate larger interfacial areas (smaller droplets) and cover the new droplets formed, hence coalescence occurred during this process.

The lower dispersal coefficients (DC) were obtained for Tween 20, meaning that the droplet size distribution curves for this surfactant were the narrower ones, although DC values for WPI stabilized nanoemulsions were not statistically different ($p \geq 0.05$). Both surfactants produced homogeneous droplet sizes. Nanoemulsions fabricated with soy lecithin presented the highest DC values, which were statistically different ($p < 0.05$) from the other surfactants, being soy lecithin the surfactant that generated the most heterogeneous droplets under the operational conditions used in this study. Oil content and emulsification pressure did not present a significant effect on the DC values. In line with these results, all distribution curves for Tween 20 presented a monomodal shape. For nanoemulsions stabilized with WPI, the shape of the size distribution curves depended on the emulsification pressure, the higher pressure generated monomodal distributions. Using soy lecithin as surfactant, only bimodal distributions were observed. This can be explained by the fact that lecithin and other phospholipids can form many kinds of self-assemblies (e.g., micelles and vesicles), which is attributed to the molecular shapes of the phospholipids according and their colloidal behavior [48–50]. By this, it is possible that the bimodal distributions consider some assemblies formed by lecithin molecules, which were unable to stabilize oil/water interfaces.

3.3. Effect of Surfactant on Z-Potential of the Emulsions

The net charge on the adsorbed emulsifier molecules determines the surface charge density and Z-potential of the droplets, which influences the magnitude of the electrostatic repulsion between droplets [51]. All nanoemulsions exhibited negative Z-potential values, which are only function of the emulsifier employed in their fabrication (Table 3). All emulsifiers presented significant differences ($p < 0.05$); but no effect of the operational conditions was found.

Table 3. Z-potential (mV) of the emulsions stabilized with the three surfactants studied.¹

Oil Content (% by Weight)	Emulsification Pressure (Bar)	Tween 20	Whey Protein Isolate	Soy Lecithin
30	500	-41.3 ± 2.2 ^{aA}	-56.7 ± 4.0 ^{bA}	-88.3 ± 4.0 ^{cA}
30	1000	-43.6 ± 1.2 ^{aAB}	-54.7 ± 1.7 ^{bAB}	-77.4 ± 1.4 ^{cAB}
50	500	-43.6 ± 0.2 ^{aAB}	-55.7 ± 2.7 ^{bAB}	-76.1 ± 0.4 ^{cAB}
50	1000	-45.9 ± 1.4 ^{aB}	-50.1 ± 1.5 ^{bB}	-74.6 ± 0.2 ^{cB}

¹ Different lower-case letters indicate significant differences ($p < 0.05$) between surfactants. Different capital letters indicate significant differences ($p < 0.05$) between process conditions.

All samples presented Z-potential values lower than -30 mV, so these nanoemulsions could be considered stable in terms of colloidal interactions against coalescence [1,37]; however, these results cannot guarantee nanoemulsion stability, since there are other factors that affect the emulsion physical stability, like droplet size and distribution, “quality” of the interface, rheology of the system, etc. According to Bot et al. [52] soy lecithin stabilized emulsions at neutral conditions have a high negative charge (-50 to -60 mV), which help to prevent droplet aggregation by generating strong electrostatic repulsion, and thus, having poor physical stability.

3.4. Rheological Behavior of the Emulsions

In food emulsions, their composition, process conditions of fabrication and environment significantly affect their rheology, which in turn, impacts their functionality, stability, sensory characteristics, and applications [51,53].

The rheological behavior of nanoemulsions clearly depends on the surfactant employed to generate and stabilize the emulsion and the emulsion composition, in terms of the oil content (Figure 2). Lineal relationships between shear rate and shear stress (i.e., Newtonian behavior) were found for nanoemulsions fabricated with Tween 20 and soy lecithin, independent on the oil content used. Similar behavior was found for WPI at 30% of oil. However, non-linear relationships can be seen for WPI at 50% of oil content, showing a shear-thinning behavior. These behaviors were confirmed by the parameters obtained after the fitting of rheological data to the Power Law model (Table 4). The flow behavior index (n values) for Tween 20 and soy lecithin were always close to 1, indicating a Newtonian behavior. Same results can be seen for WPI at 30% of oil content. However, for nanoemulsions at 50% of oil stabilized by WPI, n values were lower than the unity, confirming the shear-thinning behavior of these emulsions.

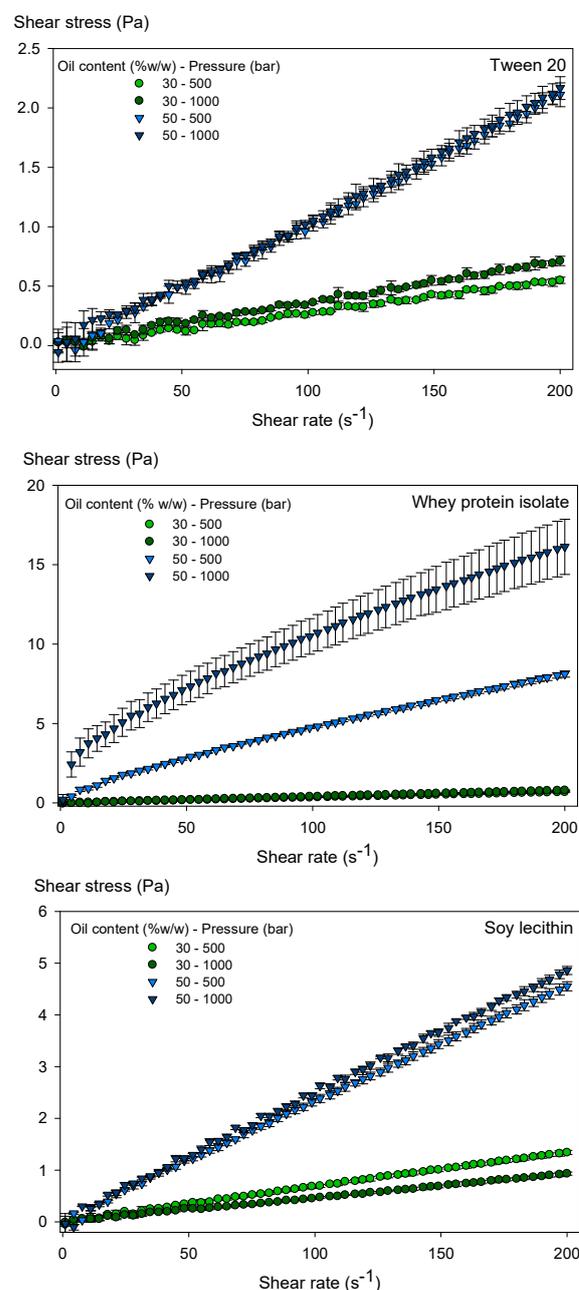


Figure 2. Rheograms for emulsions fabricated at different process conditions and stabilized by the three surfactants studied. Error bars represent one standard deviation.

Table 4. Rheological parameters for the shear-thinning model for emulsions stabilized by the three surfactants studied. ¹

Oil Content (% by Weight)	Emulsification Pressure (Bar)	Tween 20			Whey Protein Isolate			Soy Lecithin		
		K (mPa s ⁿ)	n (-)	μ_{app} (mPa s)	K (mPa s ⁿ)	n (-)	μ_{app} (mPa s)	K (mPa s ⁿ)	n (-)	μ_{app} (mPa s)
30	500	2.59 ± 0.7 ^{aA}	1.02 ± 0.05 ^{bB}	2.71 ± 0.1 ^{aA}	3.55 ± 0.6 ^{bA}	0.99 ± 0.02 ^{aB}	3.43 ± 0.2 ^{bA}	7.41 ± 1.4 ^{aA}	0.98 ± 0.03 ^{bB}	6.86 ± 0.4 ^{aA}
30	1000	4.85 ± 0.5 ^{aA}	0.94 ± 0.01 ^{bB}	3.79 ± 0.2 ^{aA}	3.47 ± 0.8 ^{bA}	1.03 ± 0.04 ^{aB}	3.88 ± 0.3 ^{bA}	4.72 ± 0.8 ^{aA}	1.00 ± 0.03 ^{bB}	4.66 ± 0.2 ^{aA}
50	500	7.23 ± 1.7 ^{aA}	1.08 ± 0.04 ^{bAB}	9.67 ± 0.8 ^{aB}	143.7 ± 20 ^{bA}	0.76 ± 0.03 ^{aAB}	53.6 ± 0.6 ^{bB}	27.91 ± 0.5 ^{aA}	0.96 ± 0.01 ^{bAB}	23.9 ± 0.4 ^{aB}
50	1000	9.04 ± 0.3 ^{aB}	1.03 ± 0.01 ^{bA}	10.34 ± 0.3 ^{aC}	866.3 ± 269 ^{bB}	0.56 ± 0.06 ^{aA}	135.8 ± 17 ^{bC}	32.26 ± 1.5 ^{aB}	0.95 ± 0.01 ^{bA}	25.9 ± 0.4 ^{aC}

¹ Different lower-case letters indicate significant differences ($p < 0.05$) between surfactants. Different capital letters indicate significant differences ($p < 0.05$) between process conditions.

As mentioned, the viscosity (or apparent viscosity) of emulsions depends on several factors. One of the major factors influencing the rheological properties of colloidal dispersions is the packing of the particles within the system [54]. It is known that apparent viscosity increases moderately with increasing disperse phase volume fraction for semi-dilute systems, because particles became to interact with each other, but apparent viscosity increases steeply when the emulsion becomes concentrated (above 60% of dispersed phase) and the droplets are packed [51,54]. The former behavior is clearly observed qualitatively in Figure 2 and quantitatively in Table 3, where the increase in the dispersed phase from 30% to 50% increases μ_{app} at 60 (s⁻¹) values for all emulsions.

In this work, the type of surfactant affected the size distributions of droplets after homogenization (Table 2). The apparent viscosity of emulsions increases as the droplet size decreases. However, viscosity of an emulsion is proportional to the viscosity of the continuous phase. Thus, the nature of the continuous phase used to formulate an emulsion often has a pronounced impact on the rheology of the system [51,54]. These factors can explain the differences in the rheology between emulsions stabilized by Tween 20 and WPI, although both kind of emulsions presented similar droplet size distributions. The apparent viscosity of WPI dispersions is higher than Tween 20 at the same concentration, due to the molecular weight difference between these two emulsifiers. Moreover, Z-Potential has an influence on emulsion viscosity. When there is a relatively strong repulsion or attraction between the droplets, their effective volume fraction may be much greater than their actual volume fraction, which leads to a large increase in emulsion viscosity [54]. This fact can explain why the apparent viscosity for soy lecithin stabilized emulsions was higher than Tween 20 stabilized emulsions, although the molecular weight of soy lecithin is lower than Tween 20, Z-Potential for soy lecithin emulsions was almost twice the value obtained for Tween 20 stabilized emulsions.

3.5. Physical Stability of the Emulsions

Physical stability of emulsions refers to the ability of emulsions to resist changes in its physicochemical properties over time [54]. The instability of emulsions may result in some undesirable effects in food including sedimentation, flocculation, coalescence and, in most extreme cases, separation of phases, which decrease the product quality and shorten shelf-life [53,54]. Therefore, its evaluation is critical in food systems. The physical stability of nanoemulsions was evaluated by means of the Turbiscan stability index (TSI). As mentioned before, TSI values consider all destabilization processes occurring in the emulsions, thus the lower TSI values correspond to the more stable system.

For the three surfactants used, the TSI values increased with storage time for all experimental conditions, indicating that these nanoemulsions are prone to physical destabilization (Figure 3). Clearly, soy lecithin was the surfactant with the highest physical instability, whereas Tween 20 and WPI showed similar values for TSI.

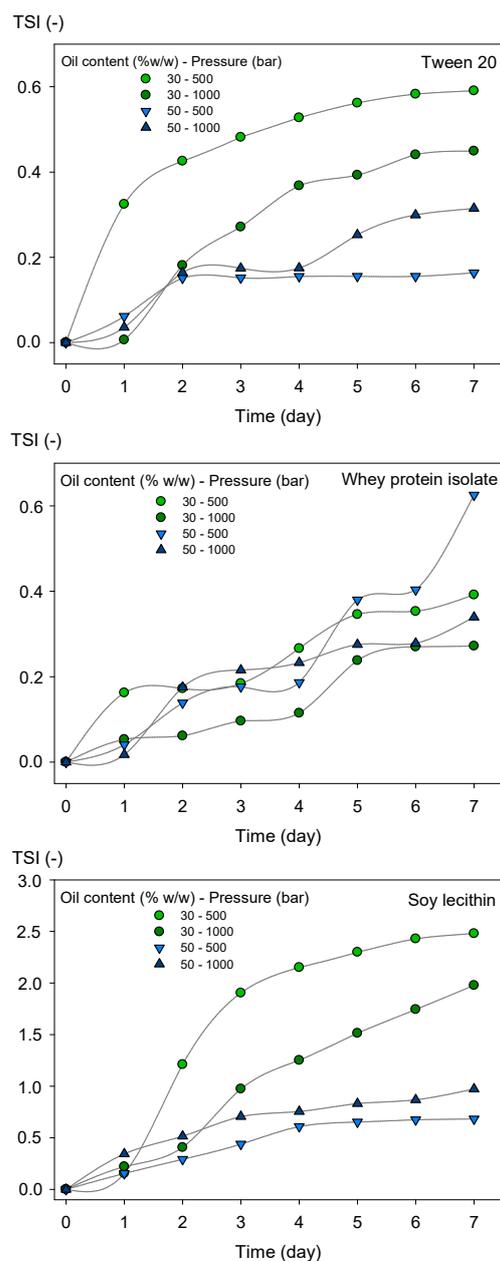


Figure 3. Turbiscan stability index (TSI, dimensionless) of emulsions fabricated at different process conditions and stabilized by the three surfactants studied. Lines are only guide for the eye.

In terms of their effect on emulsion stability, surfactants most provide repulsive forces strong enough to prevent droplets aggregation [45]. In this sense, Tween 20 is a non-ionic surfactant that generates stability due to high surface activity and short-range repulsive forces, such as steric overlap, which prevents droplets from aggregation and coalescence [55]. Nanoemulsions stabilized with Tween 20 presented low TSI values during storage under refrigerated conditions (5 °C), probably because of their small droplet diameters and homogeneity of the droplets (Table 2), in addition with the negative values of Z-potential (Table 3). However, not only the chemical nature of the surfactant affects the physical stability of emulsions. The droplet diameter and the rheology of the continuous phase are critical factors when emulsion stability is evaluated. WPI-stabilized nanoemulsions presented the best physical stability after one week of storage. It is known that whey proteins can generate a viscoelastic film around oil droplets [56,57], preventing coalescence. Diameters of droplets for nanoemulsions stabilized by WPI were similar to

those of the Tween 20-stabilized emulsions (Table 2); but the Z-potential values were lower (more negative, Table 3) and the rheology of the emulsions was different (Figure 2; Table 4). Higher apparent viscosity of the continuous phase of emulsions prevents creaming [51,54] due to the higher resistance of droplets movement, improving the stability of WPI nanoemulsions.

According to Nash and Erk [55] the high capacity of lecithin (zwitterionic surfactant) to stabilize nanoemulsions is due to the self-assembly of the molecules at the O/W interface and formation of a viscoelastic film by means of hydrogen bonds between phosphate groups, preventing coalescence. Although nanoemulsions stabilized by soy lecithin showed the lowest Z-potential values, they presented the larger diameters (D_{50} and D_{90}) and low values for apparent viscosity compared to nanoemulsions stabilized by Tween 20 and WPI, respectively. Probably, this fact caused the faster destabilization of soy lecithin nanoemulsions and the main mechanism of destabilization was flocculation and creaming and not coalescence.

4. Conclusions

This study compared the use of one synthetic surfactant (Tween 20) and two natural surfactants (WPI and soy lecithin) in the fabrication, physicochemical properties and stability of food grade nanoemulsions. The properties studied and physical stability of nanoemulsions were depending on both surfactants used and oil content. Although Tween 20 was the most effective surfactant in decreasing interfacial tension, WPI generated droplets of the same size, suggesting that WPI was more effective avoiding coalescence during the homogenization process. The highly negative Z-potential values obtained for soy lecithin nanoemulsions impacted their rheology, generating more viscous nanoemulsions in comparison with Tween 20. However, Z-potential values found for soy lecithin coated droplets do not ensure the physical stability of nanoemulsions, as concluded from their TSI results obtained. Nanoemulsions fabricated with WPI were the most stable emulsions, probably because to their small droplet size and high apparent viscosity of emulsion. WPI could be a good replacement for the development of food nanoemulsions instead synthetic emulsifiers, like Tween 20, giving the same level of physical stability but with more “creaminess” due to higher apparent viscosity.

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