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Relationship between HLB Number and Predominant Destabilization Process in Microfluidized Nanoemulsions Formulated with Lemon Essential Oil

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Featured Application: The knowledge obtained in this work could be applied to food products based on nanoemulsions and nanoemulgels, i.e., systems where the surfactant has a crucial role.

Abstract: Lemon essential oil (LEO) is associated with a multitude of health benefits due to its anticancer, antioxidant, antiviral, anti-inflammatory and bactericidal properties. Its drawback is that it is very sensitive to oxidation by heat. For this reason, researchers are increasingly investigating the use of LEO in nanoemulsions. In this work, we used laser diffraction, rheology and multiple light scattering techniques to study the effects of different HLB numbers (indicating different mixtures of Tween 80 and Span 20) on the physical stability of nanoemulsions formulated with LEO. We found that different HLB numbers induced different destabilization mechanisms in these emulsions. An HLB number lower than 12 resulted in an Ostwald ripening effect; an HLB number higher than 12 resulted in coalescence. In addition, all the developed nanoemulsions exhibited Newtonian behavior, which could favor the mechanism of creaming. All emulsions exhibited not only a growth in droplet size, but also a creaming with aging time. These findings highlight the importance of selecting the right surfactant to stabilize nanoemulsions, with potential applications in the food industry.

Keywords: microfluidization; lemon essential oil; HLB number; food emulsions

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1. Introduction

It is well-known that oil-in-water (O/W) emulsions are thermodynamically unstable systems composed of oil droplets dispersed in an aqueous continuous phase. However, kinetically stable emulsions can be produced using optimized formulation and processing techniques [1]. Emulsions are subject to a number of destabilization processes such as creaming, phase inversion, coalescence and Ostwald ripening (OR). Coalescence and OR both promote the same phenomenon in emulsions, i.e., an increase in droplet size with aging time. However, the mechanisms of the two processes are very different. Ostwald ripening involves a diffusive transfer of the dispersed phase from smaller to larger droplets; coalescence is due to the rupture of the thin film between droplets, which causes them to fuse [2]. It is important to distinguish between these two mechanisms because their causes—and possible solutions—are different. According to the Lifshitz–Slyozov–Wagner theory, Ostwald ripening depends on the interfacial tension between the two phases and on the solubility of the oil in the continuous phase. Interfacial tension and solubility both vary with the nature and concentration of the surfactant [3]. The choice of surfactant is therefore crucial when seeking to stabilize this type of system. The Ostwald ripening rate is also influenced by the size of the droplets, so that the rate increases as droplet size decreases.

One way to characterize the surfactants used in emulsions is by using the hydrophilic–lipophilic balance (HLB) number. This parameter is a semi-empirical scale used for selecting surfactants and developing emulsions. For example, a low HLB number means that a surfactant is soluble in oil; a high HLB number indicates water solubility. Sometimes, a single surfactant is not enough to obtain the stability required [4] and so a mixture of surfactants can be used to achieve synergic effects [5,6]. Interestingly, this may be a consequence of non-ideal mixing effects in the aggregates, which leads to a reduction in interfacial tension. An optimum HLB value for a selected oil phase can be obtained by using an appropriate mixture of surfactants. For example, it is common to use mixtures of Span 20 and Tween 80 to obtain different HLB numbers. Span 20 is a non-ionic surfactant which is also known as sorbitan laurate. It is used as a food additive (E493) and has an HLB number of 8.6. Tween 80 is also a food additive (E433), but it has an HLB number of 15. The use of these two surfactants in combination has been reported in several studies on food products [7,8].

Essential oils (EOs) are aromatic compounds, some of which are associated with biomedical benefits due to their anti-inflammatory, anticancer, antifungal and antioxidant properties. Lemon essential oil (LEO) is extracted from lemons mainly by cold pressing or steam distillation. LEO is known to exhibit anticancer, antioxidant, antiviral, anti-inflammatory and bactericidal properties [9,10], and has been used as a safe additive in foods, medicines and nutritional supplements [10,11]. However, LEO is very sensitive to light, temperature and oxidation. To overcome these limitations, researchers are increasingly investigating the use of LEO in nanoemulsions.

Finally, the physical stability of emulsions depends not only on their formulation, but also on the emulsification method used. However, the emulsification process is, itself, affected by the formulation used. For example, to create droplets via a shearing process, the ratio between the dispersed phase viscosity and the continuous phase viscosity cannot have a value greater than five, and the optimum ratio is approximately one [12]. Many methods are used to develop nanoemulsions using high-energy approaches, such as microfluidization. The Microfluidizer® device is based on microchannels through which a coarse emulsion is forced to pass using high pressure (up to 150 MPa). This results in a very high shearing action, producing fine emulsions. The findings of some studies indicate that microfluidization has advantages over traditional emulsifying techniques; for example, the droplet size distributions obtained are narrower and smaller [13,14]. Contrarily, the undesirable phenomenon of overprocessing is usually the result of using microfluidizers [15].

To the best of our knowledge, research into the development of emulsions formulated with lemon essential oil using a microfluidizer has been very limited to date [16]. Nonetheless, a number of studies have demonstrated the suitability of microfluidizers for the preparation of very fine emulsions containing essential oils [17–19].

In the current study, laser diffraction, multiple light scattering (MLS) and rheology were used to determine the predominant destabilization process for emulsions containing lemon essential oil with different ratios of Tween 80/Span 20, i.e., different HLB numbers. Our results highlight the important role played by surfactants in promoting the destabilization process in food emulsions.

2. Materials and Methods

2.1. Materials

Span 20 (Sorbitan laurate; density: 1.032 g/mL), which is a 100% bio-based non-ionic surfactant, and Tween 80 (Polyethylene glycol sorbitan monooleate; density: 1.07–1.09 g/mL) were used as emulsifiers. These were supplied by Sigma Aldrich. Pure lemon essential oil was purchased from Bidah Chaumel. Every material was used as received.

2.2. Methods

2.2.1. Development of Nanoemulsions

The formulation used was 0.5 wt.% of emulsifier(s), 5 wt.% of lemon essential oil, and deionized water. Different ratios of Tween 80/Span 20 were studied, with resulting *HLB* numbers of 11, 12, 13, 14 and 15. The *HLB* number was calculated as follows:

$$HLB = 15\ X_{Tween80} + 8.6\ (1 - X_{Tween80}) \quad (1)$$

where $X_{Tween80}$ is the mole fraction of Tween 80.

The coarse emulsion (250 g) was prepared as follows: Firstly, the emulsifiers Tween 80 (HLB 15) and Span 20 (HLB 8.6) were added to the deionized water. Next, the lemon essential oil was added to the aqueous phase using an Ultraturrax T-50 homogenizer at 2000 rpm for 40 s. Finally, the sample was homogenized using the same rotor–stator homogenizer (Ultraturrax T-50) at 4000 rpm for 90 s. Subsequently, the droplets formed were reduced using a microfluidization device (Microfluidizer M110P, Microfluidics, Westwood, MA, USA) at 25000 psi with a Y + Z configuration. The scheme of microfluidization was previously reported by Jafari, 2019 [15]. The Y + Z configuration consists of an interaction chamber F12Y (diameter: 75 µm) and an interaction chamber H30Z (diameter: 200 µm). Finally, the samples were kept under temperature-controlled equipment at 25 °C.

2.2.2. Laser Diffraction Results

For the nanoemulsions containing lemon essential oil, a Malvern Mastersizer 2000 (Malvern, Worcestershire, UK) was used to characterize droplet size distribution and growth in droplet size with aging time. The limit of detection was 200 nm and the refraction index used for the lemon essential oil was 1.473. The Sauter mean diameter ($D_{3,2}$) was used to characterize the nanoemulsions, calculated as follows:

$$D_{3,2} = \sum_{i=1}^{N} n_i d_i^3 \Big/ \sum_{i=1}^{N} n_i d_i^2 \quad (2)$$

where d_i is the droplet diameter, N is the total number of droplets, and n_i is the number of droplets having diameter d_i.

The samples were diluted before the measurements.

2.2.3. Rheology

All the samples were equilibrated in the selected measurement geometry for 5 min. There was no pre-stirring prior to testing. Flow curves were carried out using a Haake MARS II rheometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a sandblasted coaxial cylinder Z-20 (sample volume: 8.2 mL, R_e/R_i = 1.085, R_i = 1 cm) to avoid slip effects. Flow curves were generated using a shear rate-controlled protocol from 0.05 to 150 s^{-1}, with a maximum of 3 min per point. The temperature was fixed at 20 °C ± 0.1 °C using a Thermo Haake Phoenix C25P temperature controller. All measurements were taken on the same day that the emulsion was prepared, and all were carried out in duplicate.

2.2.4. Multiple Light Scattering

The physical stability of the nanoemulsions was analyzed using the multiple light scattering technique (Turbiscan Lab Expert, Formulaction, Toulouse, France). The backscattering (BS) of the samples as a function of the height of the measuring cell was measured for one week at 25 °C. The influence of aging time on the BS is related to the kinetics of different destabilization processes such as creaming, flocculation, Ostwald ripening and coalescence. The Turbiscan Stability Index (TSI) is a parameter that is used to characterize and compare systems with different physical stabilities [20–22], and is calculated as follows:

$$TSI = \sum_{ij} |scan_{ref}\ (h_j) - scan_i(h_j)| \quad (3)$$

where $scan_{ref}$ and $scan_i$ are the initial backscattering value and the backscattering value at a given time, respectively, and h_j is a given height in the measuring cell. *TSI* is a way to quantify the global destabilization process considering creaming, sedimentation and droplet size growth.

2.2.5. Statistical Analysis

The results obtained by laser diffraction measurements and rheological tests were analyzed by means of a one-way analysis of variance (ANOVA) using Microsoft Excel. Every sample was measured in triplicate for the laser diffraction technique, and in duplicate for the rheological tests. All statistical calculations were carried out at a significance level of $p = 0.05$.

3. Results and Discussion

3.1. Characterization of Emulsions Developed by Microfluidization

Figure 1 shows the droplet size distributions (DSDs) of lemon essential oil emulsions as a function of HLB after preparation via microfluidization. Emulsions with HLB numbers of 11 and 12 exhibited monomodal distributions with single peaks at a droplet size of less than one micron in diameter. Emulsions with HLB numbers higher than 12 exhibited bimodal distributions. The appearance of a second peak is a clear indicator of recoalescence due to overprocessing [15]. This phenomenon is very common when microfluidization techniques are used [19]. Hence, we may conclude that a surfactant ratio with a resultant HLB higher than 12 does not effectively protect the interface, and that coalescence occurs during homogenization. A similar study that investigated the effect of HLB upon oil-in-water emulsions (also formulated with Span and Tween) found that an HLB number higher than 12 led to a reduction in zeta potential (absolute value) [23]. This fact is related to a higher tendency for droplets to aggregate. This could accelerate a destabilization mechanism, such as coalescence, with aging time. In addition, the values for Sauter diameters are presented in Table 1. In terms of this parameter, there were no significant differences between emulsions with HLB numbers of 11 and 12, and this finding was supported by ANOVA testing. However, a clear increase in the Sauter diameter was found in emulsions with HLB numbers above 12, due to the occurrence of secondary peaks. In all cases, the Sauter diameter was approximately 200–400 nm, i.e., a submicron figure.

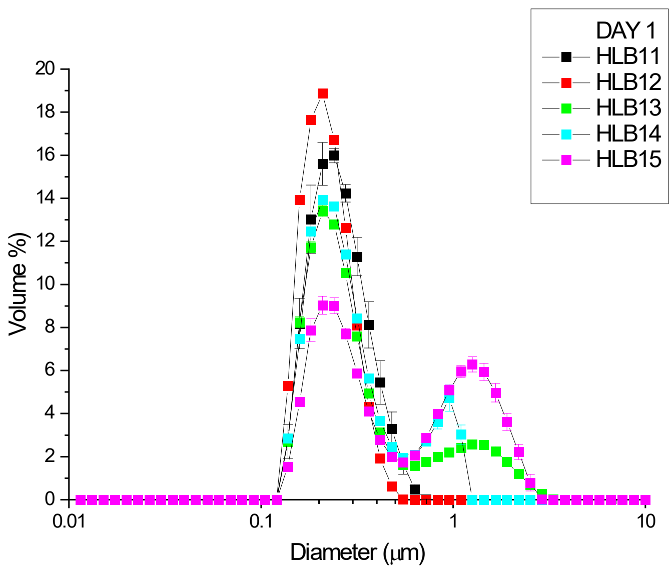

Figure 1. Droplet size distributions for emulsions formulated with 5 wt% lemon oil as a function of surfactant-mixture HLBs.

Table 1. Sauter diameter values for emulsions formulated with 5 wt% lemon oil as a function of surfactant-mixture HLBs.

HLB	11	12	13	14	15
$D_{3,2}$ (µm)	0.224 ± 0.010	0.203 ± 0.012	0.257 ± 0.034	0.246 ± 0.013	0.341 ± 0.011

Interestingly, differences in droplet size distributions did not result in any differences in flow behaviors, as can be seen in Figure 2, which illustrates the flow curves for microfluidized emulsions as a function of their resulting HLB numbers. Every emulsion developed in this study exhibited Newtonian behavior, with values for Newtonian viscosity ranging between 15.6 and 16 mPa·s. This finding indicates that viscosity does not depend on shear rate, and this is a characteristic of non-structured systems [24]. In addition, there were no significant differences between viscosity values as a function of HLB number, and this finding was also supported by ANOVA testing. Hence, we may conclude that HLB did not influence the viscosity of the samples prepared. It is well-known that viscosity in emulsions is influenced by oil concentration, droplet size distribution and the rheology of the continuous phase [24]. Consequently, if viscosity does not vary with HLB, then neither does HLB influence the rheology of the continuous phase. Thus, the viscosity was determined by the oil concentration, which was the same for all the samples, and this explains why the HLB number did not affect viscosity values.

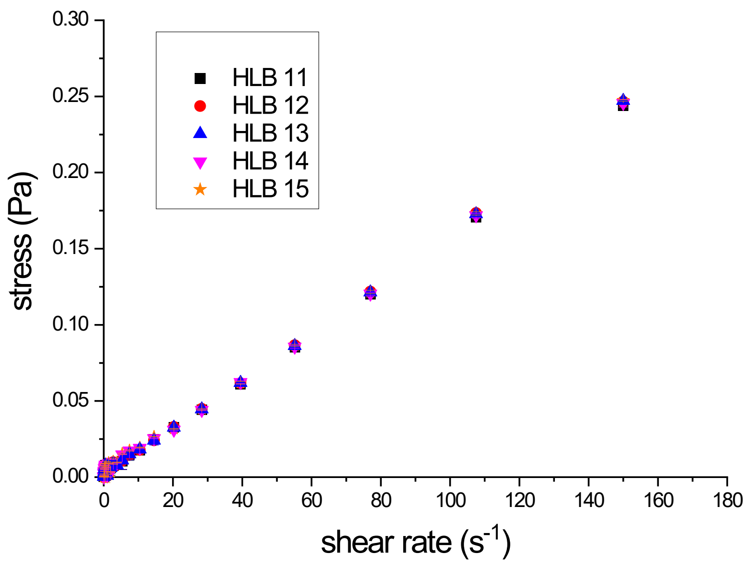

Figure 2. Flow behavior of emulsions formulated with lemon oil as a function of surfactant-mixture HLBs.

3.2. Influence of Aging Time on Microfluidized Nanoemulsions

Figure 3a–e shows the influence of aging time on droplet size distributions for emulsions with HLB numbers of 11, 12, 13, 14 and 15, respectively. All subfigures except 3b show an increase in droplet size with aging time. However, while in Figure 3a DSDs are shifted to the right along the *X*-axis, Figure 3c–e show increased second-peak heights and lower first-peak heights, indicating smaller droplets. These results point to different destabilization processes [25,26]. Specifically, the multimodal final distributions shown in Figure 3c–e are related to the phenomenon of coalescence [27]. However, Ostwald ripening is the predominant mechanism illustrated in Figure 3a because there is a shift along the *x*-axis and no second peak is evident [28]. Hence, we may say that the HLB number is a determining factor not only for droplet size distribution in emulsions after preparation, but also for the predominant destabilization mechanism which they undergo. In addition, those emulsions that exhibit bimodal distributions just after preparation due to recoalescence also undergo coalescence with aging time. Hence, the HLB number can induce one of two destabilization mechanisms due to the initial DSD produced. However, no change was observed in the emulsion prepared with an HLB of 12 (Figure 3b). Indeed, the emulsion with an HLB number of 12 (monomodal DSD after preparation) appeared to be stable with aging time in terms of droplet size, while the other systems exhibited destabilization.

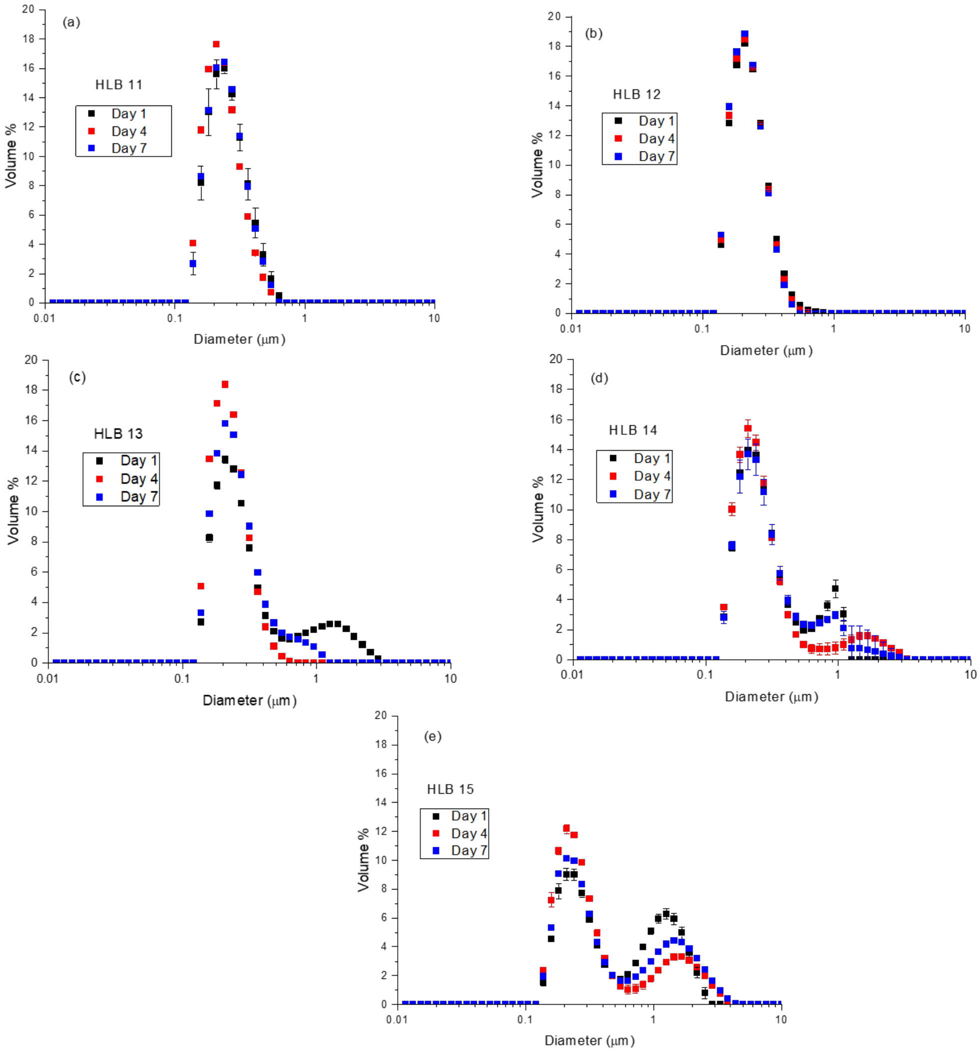

Figure 3. Droplet size distributions for emulsions with HLBs of (**a**) 11, (**b**) 12, (**c**) 13, (**d**) 14 and (**e**) 15 as a function of aging time.

To gain a deeper insight into physical stability, backscattering (BS) measurements for the emulsion with an HLB number of 11 are shown in Figure 4. Decreases in BS can be observed, particularly in the lower and higher zones of the measuring cell. These are related to a clarification process in the lower zone and to the migration of droplets in the higher zone. These observations clearly indicate the destabilization mechanism of creaming, which is promoted by the low viscosity value exhibited by the sample. This is confirmed by the Newtonian behavior of the systems developed, which indicate the total lack of structure of the sample. In addition, there is little decrease in BS in the middle zone of the measuring cell. This is related to an increase in droplet size with aging time, which is corroborated by the laser diffraction results presented above. A similar tendency in BS was observed for the other emulsions developed, but to lesser or greater extents.

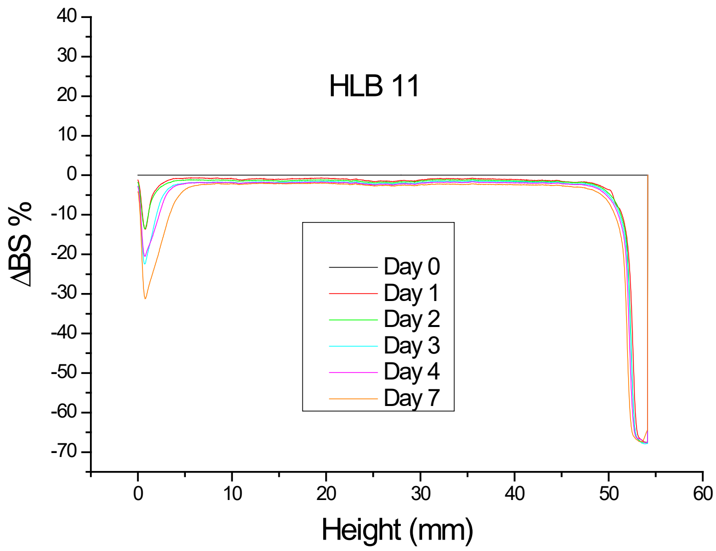

Figure 4. Variation of BS with aging time as a function of height of the measuring cell for the nanoemulsion with an HLB number of 11.

Finally, in order to characterize physical stability, and to quantify the destabilization processes affecting these nanoemulsions (creaming, Ostwald ripening or coalescence), we calculated the Turbiscan Stability Index using Equation (3) in the Materials and Methods section above. Figure 5 illustrates the Turbiscan Stability Index (TSI) for increasing aging times as a function of the HLB number. It is important to note that every destabilization mechanism contributes to the TSI, i.e., not only the growth of droplets but also creaming. Hence, the TSI values are a method to quantify all of the destabilization mechanisms involved in the dispersed systems. Interestingly, emulsions with an HLB number higher than 12, which exhibited coalescence, had higher TSI values than emulsions with HLB numbers of 12 or 11. The destabilization of these emulsions was also observed using the multiple light scattering technique. The highest TSI value was recorded for the emulsion with an HLB number of 15. This finding supported the laser diffraction results and, in this case at least, confirmed the theory that physical stability is determined by the initial DSD. However, the TSI results also revealed that the emulsion with an HLB number of 12 exhibited the greatest physical stability, confirming not only the laser diffraction results, but also the results obtained using the MLS technique.

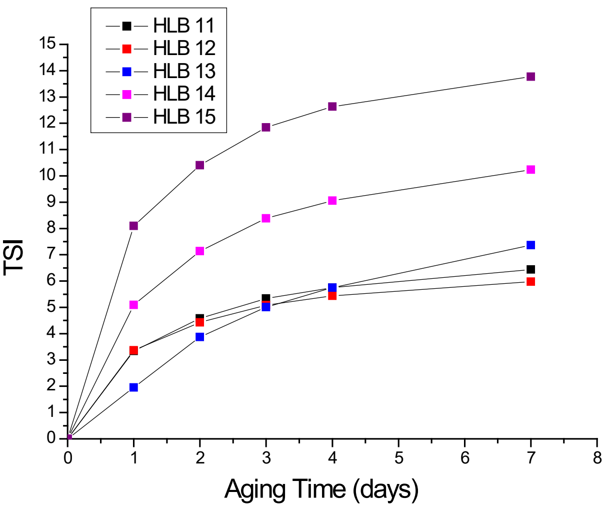

Figure 5. Variation of TSI with aging time as a function of HLB number for emulsions formulated with lemon essential oil.

4. Conclusions

In this study, oil-in-water nanoemulsions formulated with lemon essential oil were developed using various mixtures of surfactants (Tween 80 and Span 20) as an emulsifying agent. These different mixtures resulted in different HLB numbers.

The emulsion with an HLB number of 11 exhibited a monomodal droplet distribution and an Ostwald ripening phenomenon with aging time. Contrarily, emulsions with an HLB number of 13 or above exhibited a bimodal droplet distribution and coalescence with aging time. The emulsion with an HLB number of 12 exhibited a monomodal distribution and remained stable during the period studied. These findings indicated that DSDs (monomodal or bimodal) and destabilization mechanisms (coalescence or Ostwald ripening) were both influenced by the HLB number. It appears that an HLB number lower than 12 promotes Ostwald ripening, while an HLB number higher than 12 promotes coalescence.

All of the nanoemulsions developed exhibited Newtonian behavior, with viscosity values lower than 16 mPa·s. Hence, these systems revealed a lack of a microstructure. These findings indicated that a creaming process could take place because the droplets could freely move. By using the MLS technique, the occurrence of not only coalescence/OR but also of creaming was highlighted in these systems. However, a study of the physical stability of the emulsions suggested that the creaming process was least evident in the emulsion with an HLB number of 12. This may be due to the monomodal distribution presented by this system and the stable droplet size distribution with aging time observed.

In conclusion, we may say that, using the formulation described above, emulsions with an HLB number of 12 not only exhibit the best droplet size distribution but also the greatest physical stability, with no growth in droplet size and low creaming, which is extremely important for food emulsions. Such lemon essential oil nanoemulsions—with their antibacterial properties—might therefore be used as matrices for food products such as sauces, dressings and beverages. In this study, we sought to gain new knowledge about nanoemulsions formulated with lemon essential oil. Our findings highlight how different HLB numbers may influence the physical stability of food nanoemulsions.

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