Research Article

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Elaboration and characterization of nanoemulsion with orange essential oil and pectin

Antonio Sanchez, María Carmen García, María José Martín-Piñero, José Muñoz and Maria-Carmen Alfaro-Rodríguez*

Abstract

BACKGROUND: Nanoemulsions formulated with citric essential oils are currently of interest because of their physical and chemical properties and multiple applications in areas such as the food industry or agrochemicals. These are thermodynamically unstable and have almost Newtonian flow behaviour, but a suitable formulation allows systems to be obtained with good physical stability and rheological properties. The addition of pectin makes this possible. In this work, food nanoemulsions formulated with pectin, orange essential oil (5 wt%), and Tween 80 were obtained by microfluidization. First, the effect of Tween 80 concentration from 1 to 5 wt% on emulsions without pectin was evaluated. Then, pectin was added to the most stable nanoemulsion obtained and two variables were studied: the pectin solution concentration (from 2 to 6 wt%) and the pectin/emulsion ratio (1:1 or 2:1) at a fixed pectin concentration.

RESULTS: Rheological, laser diffraction, and multiple light scattering techniques were employed to determine the content of Tween 80 that results in the most stable nanoemulsion without pectin, which was 3 wt%. In addition, these techniques were used to determine the structure and physical stability of the nanoemulsions containing orange essential oil and pectin. The results obtained showed that the emulsions containing 2 wt% pectin were destabilized before 24 h. Furthermore, the emulsion with 6 wt% pectin and a 2:1 pectin/emulsion ratio showed the highest viscosity and the lowest mean diameters, and therefore the greatest stability.

CONCLUSION: This work extends the knowledge of formulation of nanoemulsions and using essential oils.

Keywords: nanoemulsion; orange essential oil; pectin; microfluidization; multiple light scattering; laser diffraction

INTRODUCTION

Interest in the use of biopolymers, such as pectin, as gelling or thickening agents has increased in the formulation of food emulsions owing to natural sources such as apple pomace, sugar beet pulp, and citrus peel.[1] Pectin is an anionic polysaccharide constituted of galacturonic acid, specifically α-1,4-linked D-galacturonic acid.[2] Specifically, pectin is a multiblock biocopolymer whose chemical structure is formed by homogalacturonan, xylogalacturonan, apiogalacturonan, rhamnogalacturonan II, and rhamnogalacturonan I, as several researchers have reported.[3,4] Commercially, pectin can be found in two forms: as high methoxyl pectin with a degree of methylation >50% or as low methoxyl pectin with a degree of methylation <50%.[5] As already mentioned, the many applications of pectin are related to its ability to modify the rheological properties of samples.[6] These properties depend on its concentration. Aqueous solutions of pectin at low concentrations show a Newtonian behaviour whose viscosity increases as the pectin concentration rises. This behaviour is a consequence of the fact that the pectin molecules are a great distance from each other and barely interact. However, pectin solutions containing a concentration >3 wt% exhibit a plateau Newtonian zone at low shear rate but a shear thinning behaviour at high shear rate.[2] These characteristics make its use in a dispersed system very interesting since it allows the delay to destabilization mechanisms such as creaming, flocculation, or coalescence. This is due to the presence of a biopolymer in the continuous phase provoking an increase in its viscosity, which reduces droplet mobility.[7] Furthermore, several studies reveal that pectin could act as an emulsifier as a consequence of its hydrophobic domains in its chemical structure that allow its absorption at the oil–water interface.[8,9]

All the pectin properties mentioned support its use in emulsion formulation because they contribute to its structural stability and additionally impart desirable texture to the final product.

* *Correspondence to: Maria-Carmen Alfaro-Rodríguez, Departamento de Ingeniería Química, Escuela Politécnica Superior, Universidad de Sevilla, C/ Virgen de Africa 7, E41011 Sevilla, Spain, E-mail: alfaro@us.es*

Departamento de Ingeniería Química, Escuela Politécnica Superior, Universidad de Sevilla, Sevilla, Spain

Emulsions are widely used in a multitude of industries, such as food, pharmaceuticals, cosmetics, cleaning products, and the agrochemicals industry, where it is increasingly common to observe encapsulated controlled-release products. The food industry is increasingly incorporating emulsions with nanometric droplet sizes (50–500 nm; termed nanoemulsions) since they offer advantages over emulsions related fundamentally to stability. In addition, they simultaneously offer other important advantages for this industry, as they improve organoleptic qualities. In addition, the protection of the active ingredients (lipophilic) that make up the product is also improved, thus improving bioavailability during intake.[10] Also, the use of nanoemulsions has also experienced a huge increase in industry, as it allows a controlled application of the active ingredients while improving its topical penetration.[11]

As is known, emulsions are dispersed droplets in a continuous phase in which the thermodynamics is unstable.[12] For this reason, the use of surfactants is essential. Surfactants are amphiphilic molecules whose main function is to enhance the stability of the emulsion. They tend to be located at the oil–water interface, which prevents some processes involved in emulsion destabilization due to its influence on steric and electrostatic repulsions.[13] A widely used surfactant is Tween 80 (T80), also called Polysorbate 80, which is a safe surfactant and is approved by the EU for use in food.

Habitually, essential oils have been employed as a dispersed phase in emulsion formulations owing to their interesting antimicrobial properties in applications such as in agriculture and the food industry.[14] In this study, orange essential oil has been used. This essential oil is composed of different organic substances, such as alcohols, aldehydes, ketones, esters, and so on, with the main component of orange essential oil being a >90% concentration of limonene. Essential oils are mixtures of a large number of organic substances, the most important of which are the aromatic compounds comprised mainly of terpenes. The orange essential oil is obtained from the orange peel, in this case organic orange; that is, during fruit cultivation, no synthetic products were used to control possible pests or phytosanitary diseases or to fertilize the soil,[15] making it a friendly product. Different industrial sectors, such as cosmetics, pharmaceuticals, or food, use orange essential oil in the formulation of their products.[16,17] For example, it can be used as an antibiotic for the treatment of acne,[18] and it presents antifungal activity against *Aspergillus flavus* growth.[19] In addition, owing to the antibacterial, antioxidant, cardioprotective, and anticancer properties of essential oils,[20] and as a consequence of the current COVID pandemic, there is growing interest in its use in food products.[21]

The main objective of the work has been the elaboration and characterization of a stable nanoemulsion containing organic orange essential oil and high methoxyl pectin. In the first part of this study, the T80 concentration was evaluated for emulsions whose orange oil concentration was adjusted. Once the amount of T80 that provides the most stable emulsion was obtained, this nanoemulsion was used as a starting point to add pectin. Several pectin concentrations and the pectin/emulsion ratio were evaluated. In order to characterize the system obtained, techniques such as laser diffraction, multiple light scattering, and rheological measurements have been used.

The importance of this work lies in the interest of nanoemulsions for the food and beverage industries as a result of their unique physical–chemical and functional properties. As a consequence of the properties of citrus essential oils, such as orange essential oil, nanoemulsions with a small drop size and good kinetic stability are expected to have excellent potential for applications such as antibacterials and preservatives for the food industry. This study aims to highlight the importance of an appropriate formulation of nanoemulsions to guarantee good stability and extend the knowledge about the use of essential oils.

MATERIALS AND METHODS

Materials

Orange essential oil (5 wt%), kindly provided by Bidah y Chaumel S.L. (Altorreal, Spain) was utilized as the dispersed phase. The non-ionic surfactant T80 (HLB 15), from 1 to 5 wt%, supplied by Sigma Aldrich (Madrid, Spain), was used as an emulsifier. Sodium azide (0.1 wt%) from Panreac (Barcelona, Spain) was added to the samples to preserve them. In order to complete the emulsions formulation, Milli-Q water was used. The formulations studied were coded EX, where X is the T80 concentration expressed as percentage by weight.

In order to obtain stable and non-Newtonian emulsions, high methoxyl pectin from lemon peel (methoxylation degree 65.8%), provided by Silvateam (Valli, Italy), was used as a thickening agent.

Preparation of emulsions

As already mentioned, the emulsions (batches of 250 g) were formulated with 5 wt% orange essential oil, T80 as emulsifier (from 1 to 5 wt%), 0.1 wt% sodium azide as preservative, and Milli-Q water. The continuous phase was formed by adding the required amount of T80 along with the necessary amount of sodium azide to Milli-Q water at room temperature using a magnetic stirring plate (Stuart SM162; Scientific Laboratory Supplies, Germany) for 600 s. Subsequently, a coarse emulsion was prepared, gradually adding oil phase at 25 °C, using a high-speed rotor-stator homogenizer (Silverson L5M; Silverson Machines Ltd, Chesham, UK), for 120 s at 2000 rpm. Then, finer emulsions were obtained by microfluidization using a Microfluidizer M-110P device (Microfluidics, USA) with fixed processing parameters, namely three recirculation cycles, 17.24 MPa (2500 psi), and 25 °C.

At least two samples were prepared with each formulation.

Preparation of nanoemulsions with pectin

Based on the results obtained from the influence of weight-percentage of T80 on the properties of nanoemulsions, the most stable formulation (optimal nanoemulsion) was used as a starting point for the addition of pectin. These samples were prepared using different concentrations of pectin (2, 4 and 6 wt%) and different pectin/optimal nanoemulsion mass ratios (1:1 and 2:1) (Table 1). Pectin stock solutions (250 g baths) were prepared by gradually adding the amount of pectin powder needed to the required water mass at 700 rpm for 600 s. The gum solutions were stirred using a high-speed Ikavisc MR-D1 homogenizer (Ika, Staufen, Germany) along with a sawtooth-type impeller for 1200 s at 400 rpm at 70 °C. The systems were kept in quiescent rest until they reached room temperature and were then left to stand for 24 h at 7 °C for complete polymer hydration. The final emulsions were prepared by dispersing the gum solution in the optimal nanoemulsion using an Ikavisc MR-D1 and a helical impeller at 250 rpm for 300 s at room temperature.

Sample characterization

Droplet size distribution

The droplet size distribution (DSD) was determined by laser diffraction using a Mastersizer 2000 analyser (Malvern Instruments,

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Table 1. Formulation of nanoemulsions containing pectin

	Nanoemulsion containing 2 wt% pectin[a]		Nanoemulsion containing 4 wt% pectin		Nanoemulsion containing 6 wt% pectin	
	EG2 (1:1)	EG2 (2:1)	EG4 (1:1)	EG4 (2:1)	EG6 (1:1)	EG6 (2:1)
Orange oil (wt%)	2.5	1.7	2.5	1.7	2.5	1.7
Tween 80 (wt%)	1.5	1.0	1.5	1.0	1.5	1.0
Pectin (wt%)	1.0	1.3	2.0	2.7	3.0	4.0

[a] Ratios in parentheses indicate pectin/emulsion ratio.

Malvern, UK), associated with a wet dispersion unit that was set at the following specific conditions: 80% of pump and 50% of stirrer. The orange oil refraction index was 1.47, and for the dispersant the absorption and refraction indexes used were 0.5 and 1.33 respectively.

The particle size was expressed as the Sauter (or surface-weighted) mean diameter $D[3,2]$ and the De Brouckere (or volume-weighted) mean diameter $D[4,3]$, which are defined as follows:

$$D[3,2] = \frac{\sum_{i=1}^{N} n_i d_i^3}{\sum_{i=1}^{N} n_i d_i^2} \quad (1)$$

$$D[4,3] = \frac{\sum_{i=1}^{N} n_i d_i^4}{\sum_{i=1}^{N} n_i d_i^3} \quad (2)$$

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

To discover the polydispersity of the DSD, the 'span' was used, which was defined as follows:

$$\text{Span} = \frac{D[v,0.9] - D[v,0.1]}{D[v,0.5]}$$

where $D[v,0.9]$ and $D[v,0.1]$ represent the 90th and 10th percentiles, respectively and $D[v,0.5]$ is the median.

In order to evaluate the emulsion stability, these tests were performed at 1, 5, and 14 days of aging time.

At least two replicates of each test were performed at room temperature.

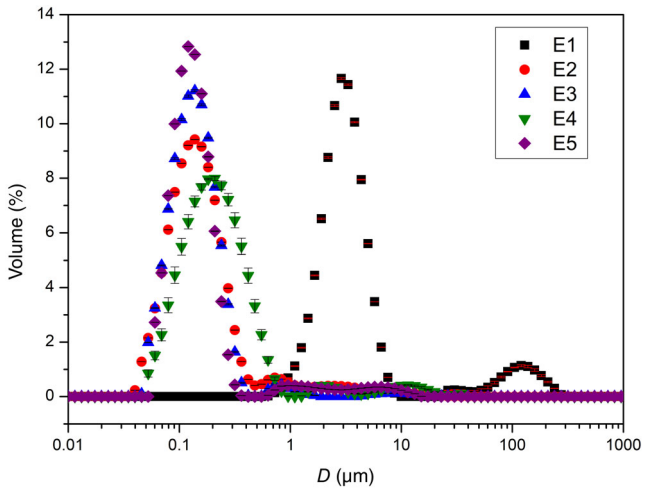

Figure 1. Influence of the Tween 80 concentration on the droplet sizes distributions of orange essential oil nanoemulsions at 24 h. Room temperature. Values are the means of at least three determinations plus/minus standard deviation.

Physical stability

The physical stability of emulsions in quiescent conditions was evaluated using a Turbiscan Lab vertical analyser (Formulaction, Toulouse, France) according to the multiple-light scattering theory. Measurements were made at room temperature, whereby the backscattering as a function of the length of the vessel containing the nanoemulsions was determined during the aging time.

Rheological characterization

To evaluate rheological properties of the nanoemulsions, steady-state measurements were performed. For this purpose, a control stress rheometer Haake Mars (Thermo-Fisher Scientific/Haake, Karlsruhe, Germany) joined to a thermostatic circulator was utilized. The tests were carried out in a shear stress range of 0.1–1.0 Pa at 25 °C using a double cone geometry whose diameters and angle were 60 mm 1°, respectively.

The viscoelastic properties of pectin-containing nanoemulsions were found by means of small-amplitude oscillatory shear measurements. For these tests, a controlled-stress AR2000 rheometer (TA Instruments, Crawley, UK) was used. First, stress sweep tests were carried out to determine the linear viscoelastic range in the shear stress range 8×10^{-3}–10 Pa at a fixed frequency of 1 Hz at 25 °C. Then, mechanical spectra were obtained from 0.05 to 10 rad s^{-1} at a particular shear stress within the linear viscoelastic range at 25 °C. Serrated parallel plate geometry was used for this point.

Each rheological measurement was performed twice with a fresh sample.

Statistical analysis

The results were expressed as means with standard deviations of at least two experimental runs. Fisher test was used to compare means with significant differences ($P < 0.05$). For all statistical analyses, OriginPro version 8 (OriginLab, Northampton, MA, USA) was used. All calculations were considered statistically significant at $P = 0.05$.

RESULTS AND DISCUSSION

Influence of T80 concentration on the properties of nanoemulsions

Figure 1 shows the influence of the T80 concentration on the DSD after aging for 24 h. As can be observed, all the systems studied showed a monomodal distribution except the emulsion containing 1 wt% T80, which exhibited a peak around 3 μm and another around 100 μm, this latter probably due to the existence of air bubbles. The rest of the emulsions are characterized by having a very similar distribution with drop sizes in the nanometric range; in particular, they exhibited a maximum population of around

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Table 2. Influence of Tween 80 concentration on the mean values of the Sauter and volumetric diameters and the span values in the aged emulsion 24 h containing 5 wt% orange essential oil

	$D[3,2] \pm$ SD (μm)	$D[4,3] \pm$ SD (μm)	Span ± SD
E1	2.710 ± 0.008	13.34 ± 0.11	2.02 ± 0.181
E2	0.120 ± 0.001	0.53 ± 0.52	2.06 ± 0.329
E3	0.120 ± 0.005	0.29 ± 0.06	1.24 ± 0.054
E4	0.150 ± 0.022	0.55 ± 0.12	1.76 ± 0.424
E5	0.150 ± 0.021	0.56 ± 0.17	2.21 ± 0.782

Values are means of at least three determinations plus/minus standard deviation (SD). Room temperature.

100 nm. $D[3,2]$, $D[4,3]$, and span are shown in Table 2. As can be observed, these parameters were different for all the formulations evaluated. However, the results obtained from the Fisher test (data not shown) demonstrated that the mean difference found in $D[3,2]$ for the E2 and E3 emulsions was not significant at the 0.05 level. Regarding $D[4,3]$, the Fisher test indicated that the mean difference was not significant at the 0.05 level in all cases.

The influence of aging time on the DSD of emulsions E2, E3, E4, and E5 is shown in Fig. 2. As can be observed (Fig. 2(b)), the DSD of E3 hardly changed in the study time, which was supported by the analysis of variance (ANOVA) results. This is due to the fact that the surfactant successfully protected the interface against every destabilization mechanism, such as coalescence or Ostwald ripening.[22] In addition, this effect could be observed for the E2 emulsion. However, the E4 emulsion (Fig. 2(c)) exhibited a decrease in the percentage of population with smaller droplets and the appearance of a second population of larger droplets with aging. This incident indicated the existence of a coalescence process. An Ostwald ripening destabilization process is discarded, since in that case the same shape of the distribution would be expected but shifted towards larger sizes.[23] The emulsion containing 5 wt% T80 also exhibited a DSD shifted toward higher diameters, especially at 15 days of aging time.

Regarding the mean diameters, it should be noted that emulsions containing 4 and 5 wt% of T80 showed significant differences with aging time. Furthermore, the values of $D[3,2]$ and $D[4,3]$ as a function of the aging time of E5 indicated an increase in the diameter of the droplets that, in view of the results shown in Fig. 2(d), could be the result of a coalescence destabilization process. Interesting, $D[4,3]$ of this emulsion at 15 days of aging (23.96 μm) was significantly higher than that exhibited for E4 at the same aging time (9.62 μm). Instead, emulsions E2 and E3 did not exhibit significant differences in mean diameters, indicating that there was no coalescence process during the study time. These results were supported by ANOVA data.

The influence of T80 concentration on the shear stress as a function of shear rate is shown in Fig. 3 at 24 h of aging time. A Newtonian behaviour can be observed for all emulsions studied. This fact was supported by the fitting parameters to the power law model, Eqn (3), shown in Table 3:

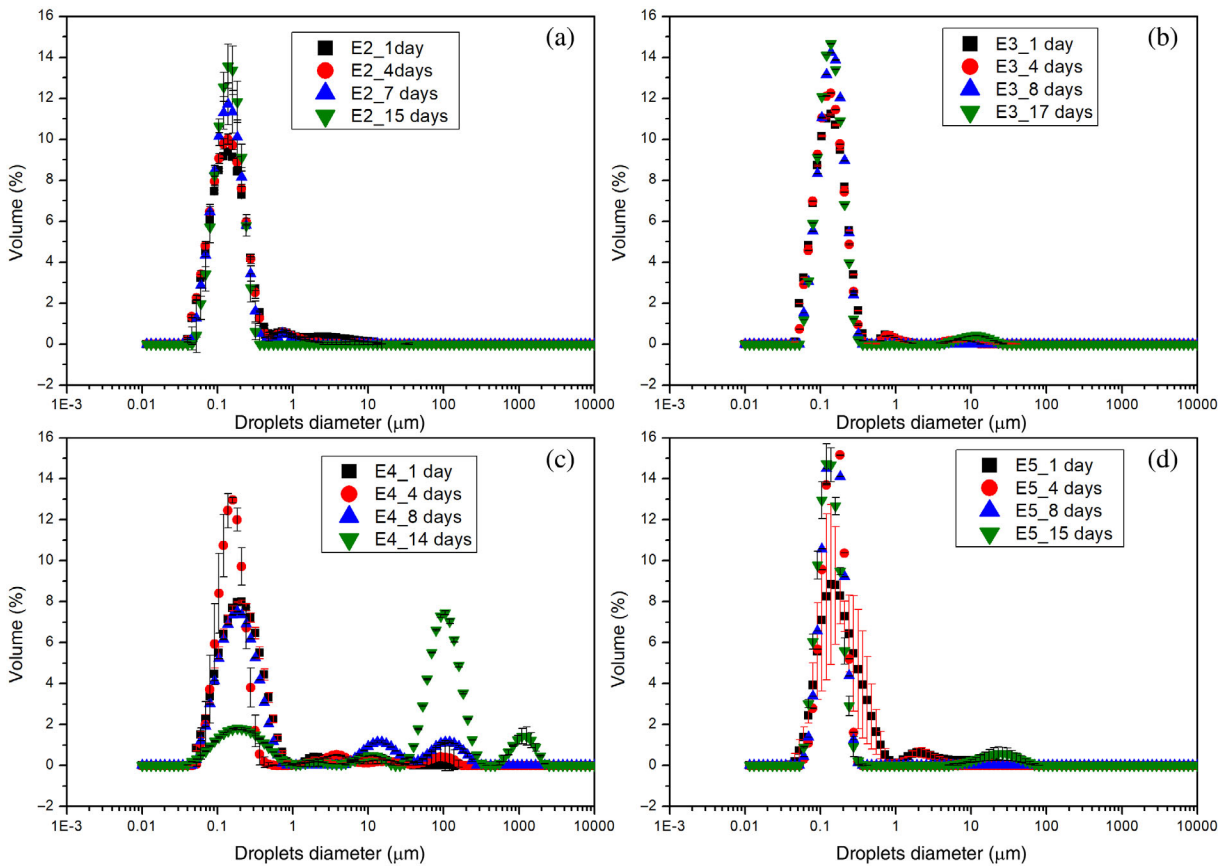

Figure 2. Influence of aging time on the droplet size distribution of orange essential oil nanoemulsions prepared with Tween 80: (a) E2; (b) E3; (c) E4; (d) E5. Values are means of at least three determinations plus/minus standard deviation. Room temperature.

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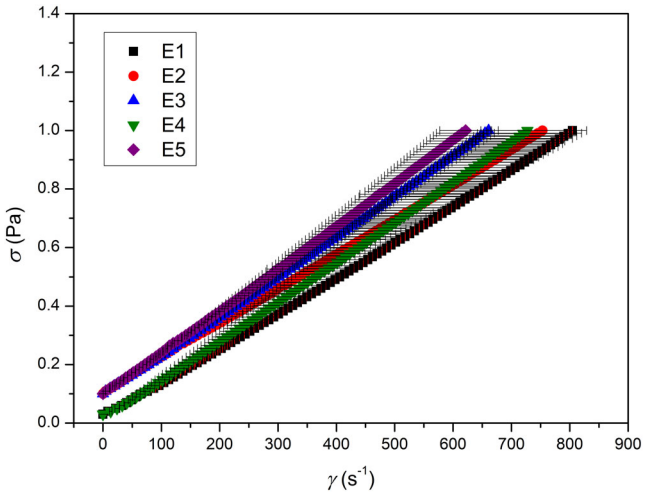

Figure 3. Influence of the Tween 80 concentration on flow curves at 24 h of orange essential oil nanoemulsions. Values are the means of two determinations plus/minus standard deviation. $T = 25$ °C.

$$\tau = k\dot{\gamma}^n \quad (3)$$

where τ is the shear stress, k is the consistency index, and n is the flow index. A trend to increase the consistency index was observed with increasing T80 concentration. Regarding the flow index, there were no trends. From ANOVA, it can be observed that both k and n means were significant at the 0.05 level.

The flow curves of the E1, E2, and E3 nanoemulsions exhibited a slight increase in viscosity with aging time (data not shown). It should be noted that an increase in the droplet concentration at the top of the sample as a consequence of a creaming destabilization mechanism could explain this fact, since this would provoke an increase in the viscosity. Emulsions containing 4 and 5 wt% T80 exhibited more complex flow behaviour with aging, which could be the result of an increase in viscosity promoted by droplet flocculation probably induced by an excess of T80 in the continuous phase and a further decrease in viscosity due to coalescence.

In order to study the destabilization mechanism of these emulsions, multiple light scattering has been used. Figure 4 shows the percentage backscattering profile in a whole-length tube as a function of the aging time of E1 and E4 emulsions by way of example.

The emulsion containing 1 wt% T80 (Fig. 4(a)) showed a decrease in the percentage backscattering signal at the bottom of the measurement cell and an increase in the upper part. This behaviour indicated the existence of a destabilization process by creaming as a consequence of the difference in density

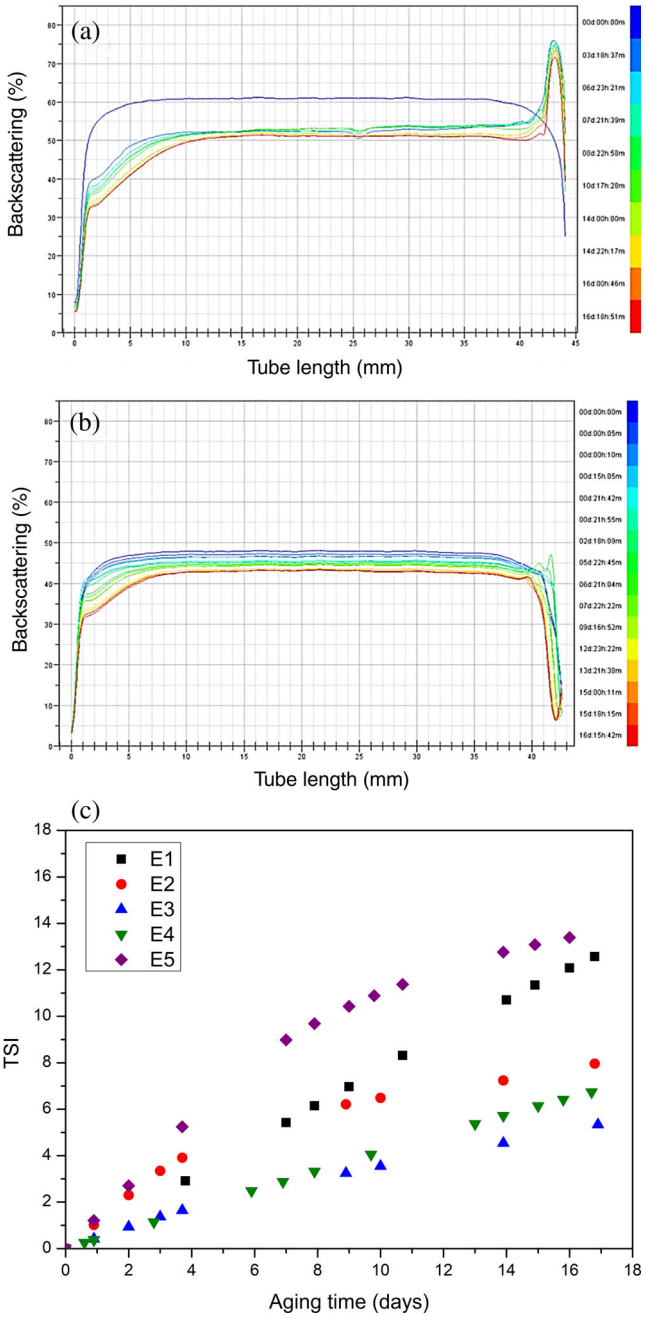

Figure 4. Backscattering profiles of nanoemulsion (a) E2 and (b) E3. (c) Turbiscan stability index (TSI) for every nanoemulsion studied containing orange essential oil. $T = 25$ °C.

Table 3. Influence of the Tween 80 concentration on the parameters of the fitting to the power law model in the emulsion containing 5 wt% orange essential oil

	k (mPa s^n) ± SD	n ± SD	R^2
E1	$0.0012 \pm 7.1 \times 10^{-5}$	0.990 ± 0.006	0.995
E2	$0.0037 \pm 2.8E \times 10^{-4}$	0.844 ± 0.023	0.999
E3	$0.0037 \pm 1.4 \times 10^{-4}$	0.861 ± 0.003	0.995
E4	0.001 ± 0	0.997 ± 0.004	0.994
E5	$0.0044 \pm 9.9 \times 10^{-4}$	0.843 ± 0.044	0.999

Values are the means of two determinations plus/minus standard deviation (SD). $T = 25$ °C.

between the continuous phase and the dispersed phase.[24] The emulsions with 2 and 3 wt% T80 (data not shown) exhibited a similar behaviour to E1, but the creaming was less significant. These results were consistent with those found in the droplet size distribution and the flow curves. At lower concentrations of surfactant (E1, E2, and E3), the high polydispersity, together with the low viscosity shown by these nanoemulsions, facilitated the migration of the droplets to the upper part of the measurement cell. The E3 emulsion, with the smallest span value, was the emulsion least affected by this phenomenon. Finally, emulsions with higher amounts of surfactant (4 and 5 wt% T80) (see Fig. 4(b)) exhibited a global decrease in percentage backscattering with aging time, which indicated an increase in droplet size as the main

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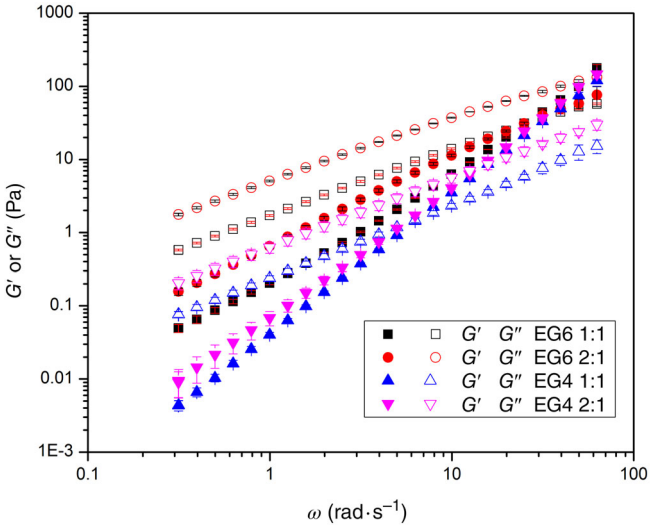

Figure 5. Influence of the pectin/nanoemulsion ratio and the pectin on the mechanical spectra of the essential orange nanoemulsions. Values are the means of two determinations plus/minus standard deviation. $T = 25$ °C.

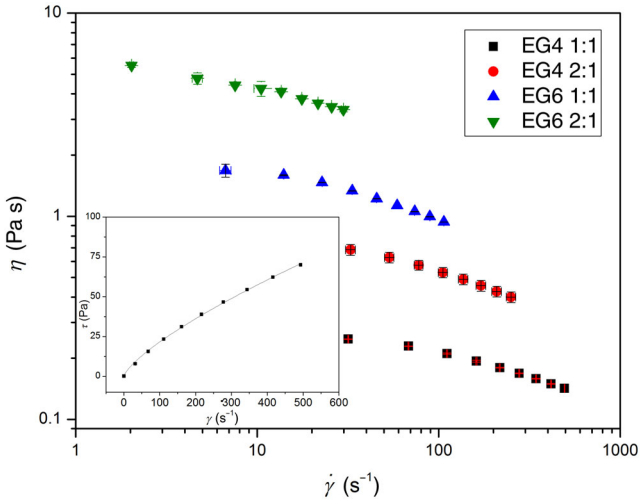

Figure 6. Influence of the pectin/nanoemulsion ratio and the pectin concentration on the flow curves of the essential orange nanoemulsions. Values are the means of two determinations plus/minus standard deviation. $T = 25$ °C.

destabilization mechanism.[25] The growth in droplet size could be attributed to a depletion flocculation process probably caused by an osmotic imbalance that resulted in the exclusion of surfactant micelles between neighbouring droplets, as reported by McClements.[26] These micelles would be formed as a consequence of an excess of surfactant. It should be highlighted that this mechanism sometimes causes coalescence due to breakdown of interfaces when flocs rise and collide. This occurred in nanoemulsions E4 and E5 according to the results obtained by laser diffraction (the volumetric mean diameter increased significantly with aging time).

Different destabilization mechanisms have been found. For this reason, to better understand the multiple light scattering data, the global Turbiscan stability index (TSI; considering all destabilization processes)[27,28] has been analysed. This parameter is defined as follows:

$$\mathrm{TSI} = \sum_j |\, \mathrm{scan}_{\mathrm{ref}}(h_j) - \mathrm{scan}_i(h_j) \,| \qquad (4)$$

where $\mathrm{scan}_{\mathrm{ref}}$ and scan_i represent the scans at $t = 0$ and $t = i$ respectively, h_j is a given length of the sample vial, and TSI is the sum of all the backscattering differences for the whole height of the measuring cell. Therefore, the higher the value of TSI is, the higher the instability is.[29] Figure 4(c) shows the TSI as a function of the aging time for every nanoemulsion studied. In this figure, we observe that there is an increase in TSI with age in all cases. However, the E3 and E4 nanoemulsions showed the lowest TSI values, and therefore the highest stability. These nanoemulsions had similar values of TSI until the ninth day, and from this day the TSI values of E3 were lower than those of E4. As a result of having the highest stability, E3 emulsion (3 wt% T80) was selected to perform the next part of the work on the addition of pectin.

Influence of pectin concentration on the properties of E3 nanoemulsion

The samples obtained by mixing pectin and E3 nanoemulsion – except those containing 2 wt% pectin, which were destabilized 24 h after production – maintain a nanometric Sauter mean diameter although slightly bigger (this diameter ranged from 220 to 330 nm) than E3 (data not shown).

Figure 5 shows the storage modulus G' and loss modulus G'' of nanoemulsions containing pectin as a function of frequency at 24 h of aging time. These oscillatory rheological results can indicate whether these systems are strongly or weakly associated. Regardless of the pectin/emulsion ratio or pectin concentration used, at low frequencies it can be observed that the values of G'' exceeded those of G' and both depend on frequency. At the highest frequencies a crossing point was observed or tended to be observed. This behaviour is typical of non-flocculated or weakly flocculated emulsions, indicating a more viscous liquid-like behaviour than elastic and little or any network structure.[30] A global comparison of all the mechanical spectra makes it possible to affirm that an increase in the pectin concentration in the stock pectin solution or an increase in the pectin/emulsion ratio caused an increase in the values of both the storage modulus and the loss modulus. This fact could be due to the increase in polymeric entanglements in pectin that form the continuous phase of the new system produced by mixing the nanoemulsion and the pectin solution.[31]

Figure 6 is a log/log plot showing the effect of increasing pectin concentration on the functional relationship between viscosity and shear rate for an orange essential oil-in-water nanoemulsion. Additionally, the inset shows the shear stress *versus* shear rate. It was possible to distinguish there was a decline of the viscosity values as shear rate values increased, which could be attributed to a certain shear-thinning behaviour. This shear thinning behaviour could be interpreted as indicating the occurrence of weak interactions between droplets, which caused a weak network.[32] In fact, in these systems, the network was very weak, and the interaction forces were easily overcome when a low shear stress was applied. The results were well fitted to the Hershel–Bulkley model (Eqn (3)), and its fitting parameters are shown in Table 4. As can be observed in this table, there was a slight increase in k as both the pectin concentration and/or pectin/emulsion ratio increased, which is directly associated with an increase of the viscosity. Furthermore, the flow index values ($n < 1$) demonstrated that these samples had a shear-thinning behaviour, with the values being higher as the pectin concentration increased. These results are in accordance with the thickening effect exerted by the pectin

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Table 4. Influence of the pectin/nanoemulsion ratio and the pectin concentration on the fitting parameters to the Hershel–Bulkley model

		$10^{17} \times \tau_0$ (Pa)	k (Pa s^n)	n	R^2
EG4	1:1	7.56 ± 1.78	0.705 ± 0.007	0.743 ± 0.002	0.999
	2:1	11.9 ± 0.990	1.937 ± 0.076	0.716 ± 0.0007071	0.999
EG6	1:1	68.5 ± 82.7	3.463 ± 0.052	0.722 ± 0.003	0.999
	2:1	20.4 ± 0.424	6.92 ± 0.023	0.787 ± 0.002	0.999

Values are the means of two determinations plus/minus standard deviation (SD). $T = 25$ °C.

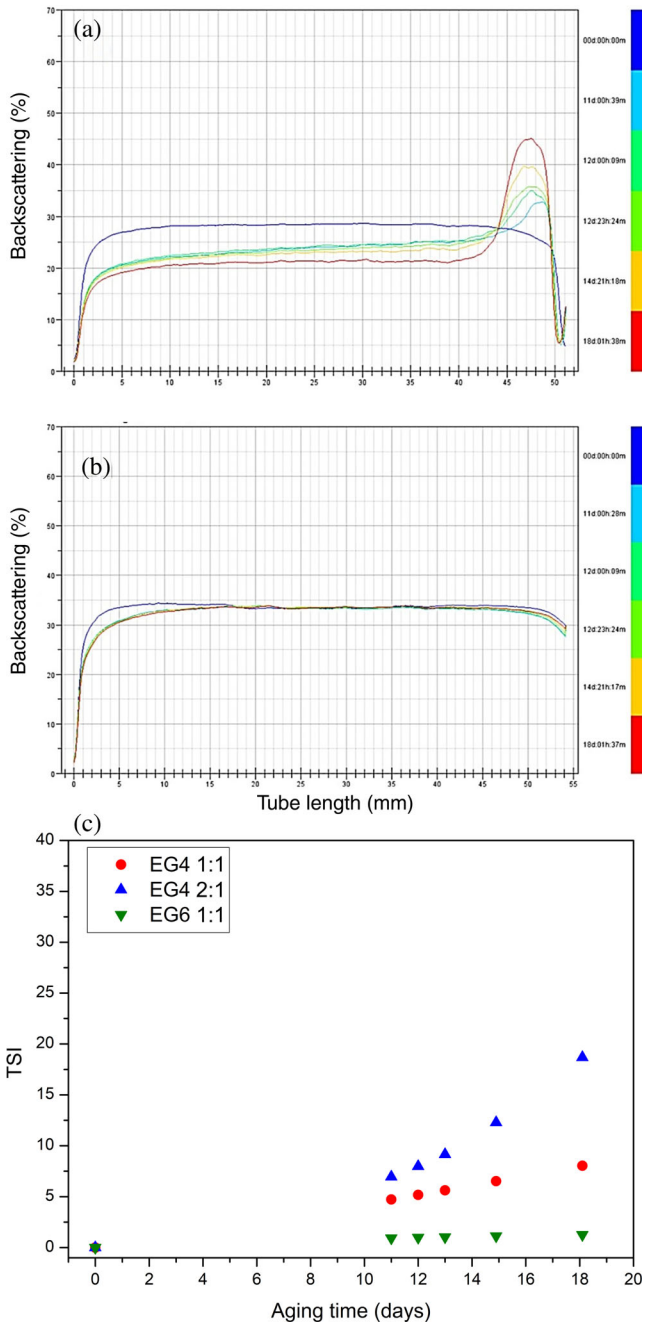

Figure 7. Backscattering profile of (a) EG4 (2:1) and (b) EG6 (2:1). (c) Influence of the pectin/nanoemulsion ratio and the pectin concentration (a) on the Turbiscan stability index (TSI) parameter of emulsions containing essential orange oil and pectin.

solution that was present in the continuous medium of these systems. This effect was related to the ability of pectin to hold a high amount of water, enhancing the viscosity. Other authors also observed this behaviour.[33]

In order to assess the stability of the pectin nanoemulsions, the multiple light scattering technique was used. Nanoemulsions containing 4 wt% pectin and pectin/nanoemulsion mass ratios of 1:1 and 2:1 (EG4 (1:1) and EG4 (2:1)) and nanoemulsion with 6 wt% pectin and a pectin/nanoemulsion ratio of 1:1 (EG6 (1:1)) showed an increase in percentage of backscattering at the top of the measuring cell from 11–12 days of aging time, whereas this parameter suffered a decline in the rest of the vessel. This behaviour is typical in samples destabilized by creaming, coalescence, or Ostwald ripening.[25] In Fig. 7(a), the results of nanoemulsion with 4 wt% pectin and pectin/nanoemulsion of 2:1 (EG4 (2:1)) are shown by way of example. Figure 7(b) shows the backscattering values for the same pectin/nanoemulsion ratio previously shown but at 6 wt% pectin (EG6 (2:1)). These results demonstrated that the pectin added as stabilizer reduced the droplet movement, and the processes of destabilization were delayed in time as a consequence of a higher stability. The TSI results (Fig. 7(c)) also showed that the stability grew as the pectin concentration and the pectin/emulsion ratio increased.

Therefore, it must be highlighted that a higher percentage of pectin in the formulation offers greater mechanical protection and protection from conditions external to the nanoemulsion, and consequently greater stability.

CONCLUSIONS

In this paper, oil-in-water emulsions containing 5 wt% orange essential oil as the dispersed phase and T80 as the emulsifier were obtained by means of a microfluidization technique. It was proved that microfluidization allows a reduction in the diameter of the droplets to be up to nanometre values, which led to an improvement in the physical stability. These emulsions exhibited an almost Newtonian behaviour whose droplet sizes, except for E1, were nanometric and showed a significant trend to increase with increasing T80 concentration. Multiple light scattering demonstrated that the nanoemulsions containing the lower emulsifier concentration studied (E1, E2, and E3) suffered a destabilization by creaming, whereas the nanoemulsions with the higher T80 concentrations (4 and 5 wt% T80) mainly suffered a depletion flocculation destabilization mechanism that led to a further coalescence process, in concordance with the laser diffraction. The most stable emulsion turned out to be the nanoemulsion with 3 wt% T80 (E3). The mix of this optimal nanoemulsion with pectin solutions of different concentrations (2, 4, and 6 wt%) in different pectin/optimal nanoemulsion mass ratios (1:1 and 2:1) increased

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the viscosity of the sample obtained, influencing their rheological properties and stability. All emulsions obtained with a stock pectin solution concentration >2 wt% had viscoelastic properties and shear-thinning flow behaviour. An increase in the pectin/emulsion ratio or pectin stock solution concentration provoked an increase in both the viscoelastic moduli values and the viscosity. These emulsions simultaneously underwent a main mechanism of destabilization by creaming and an increase in droplet size. Despite this, the addition of pectin enhanced the long-term stability of the nanoemulsion, with the most stable emulsion being the one with the highest final concentration of pectin; namely, the emulsion containing 6 wt% pectin and a pectin/nanoemulsion mass ratio of 2:1.

This study has revealed the impact of formulation on the obtaining of stable nanoemulsions produced by microfluidization containing orange essential oil. Additionally, it has developed systems with potential applications both as natural food preservatives as a result of the properties of orange essential oil and as nanocarriers of active ingredients due to the nanoemulsions.

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