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## **Emulsions of sunflower wax in pectin aqueous solutions: physical characterization and stability**

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**Abstract**

The knowledge of the stability and physical properties of film-forming solutions is necessary for optimizing the process design of films. In order to evaluate their applicability for the production of edible films, the rheological and microstructural properties, particle size and physicochemical stability of aqueous emulsions of low methoxyl pectin and sunflower waxes from normal and high-oleic hybrids were assessed. Emulsions were prepared with different pectin concentrations (1, 2 and 3% w/w) and wax proportions (0.1, 0.2 and 0.3 g/g pectin). The rheological behavior was best described by the power law model. The values of the behavior index ( $n$ ) were close to 1, exhibiting a behavior close to Newtonian fluids. The addition of waxes caused an increase in viscosity and shear stress. The particle size of the emulsions made with waxes from high-oleic sunflower was smaller than those from the normal hybrid. In most cases, size distributions with greater height and less amplitude were obtained, mainly when the pectin content was higher. Confocal images allowed to observe the presence of waxes and their dispersion in the pectin matrix. Destabilization phenomena such as sedimentation, coalescence and creaming were observed at long test times independent of the wax origin. These results evidence the potential use of these emulsions for the manufacture of edible films.

**Keywords:** physical stability, low methoxyl pectin, emulsions, particle size distribution, rheology, confocal microscopy, normal sunflower wax, high-oleic sunflower wax.

*Abbreviations:* SFW, sunflower waxes; NE, emulsion from normal sunflower wax; HOE, emulsion from high-oleic sunflower wax; BS, backscattering; S, span.

## 1. Introduction

In recent years, the interest and concern about the environmental impact of synthetic packaging waste, as well as increasing consumer demand for safe, high-quality convenient foods with long shelf life have boosted the research of alternatives focused on edible packaging or films formulated with materials with unique properties (Janjarasskul & Krochta, 2010). These edible films are generally made from one or more of the four major types of materials: lipids, resins, polysaccharides and proteins (Baldwin, Nisperos-Carriedo, & Baker, 1995), and other components derived from renewable sources. One of the strategies used to associate the properties of polysaccharides and lipids is the elaboration of emulsions, which allow the dispersion of the hydrophobic component in an aqueous matrix. Obtaining film-forming solutions is also industrially interesting because it involves few operations in the film preparation (Pérez-Gago & Krochta, 1999) and it allows the complementary association of the functional properties of each component and minimizes their disadvantages (Debeaufort, Quezada-Gallo, & Voilley, 1998). Pectin is one of the polysaccharides most commonly used in edible film formulation, and it is capable of reducing the interfacial tension between an oil phase and a water phase, a property that makes it efficient in emulsion preparation (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). Sunflower waxes (SFW) are natural lipid barriers that are useful and effective in reducing water vapor transfer between food and the environment.

The knowledge of the rheological properties of film-forming solutions or emulsions is necessary for optimizing the process design (Chen, Kuo, & Lai, 2009). The rheological behaviors vary depending on the concentration of the components and the degree of interaction between them (İbanoğlu, 2002). The size and organization of the lipid particles affect the properties of the entire emulsion, and in turn, the barrier properties of the coatings made from it (McHugh & Krochta, 1994). Microscopy techniques provide information about the structure, size and organization of the emulsion components. These techniques work with different physicochemical principles, and they can be used to examine different levels and types of structural organization of the emulsions (McClements, 1999). From a physicochemical point of view, emulsions are thermodynamically unstable systems. According to their kinetic stability, they tend to separate into two immiscible phases (Comas, Wagner, & Tomás, 2006). An alteration in the spatial distribution or structural organization of the molecules results in physical instability. Phenomena such as sedimentation, creaming, coalescence or flocculation are examples of physical instability (McClements, 1999). The latter two include particle

migration and are considered reversible processes, while the first two phenomena involve changes in particle size and are considered irreversible (Comas et al., 2006; Lemarchand, Couvreur, Vauthier, Costantini, & Gref, 2003; Pan, Tomás, & Añón, 2002). Therefore, the stability of emulsions is an important feature to determine their efficiency in obtaining edible coatings (Panchev, Nikolova, & Pashova, 2009). In a previous work (Baümler, Carelli, & Martini, 2013), some physical properties of aqueous solutions of pectin and sunflower wax prepared with different pectin and SFW contents were analyzed, evaluating their stability and rheology in order to determine their use as a basis for the development of edible coatings. In the present study, the investigation was extended to SFW from different hybrids, considering the effect of the SFW-to-pectin proportions on the emulsion, as well as their evaluation using confocal microscopy and particle size distribution.

The aim of this work was to analyze the rheological properties, particle size distribution, microstructural characteristics and physicochemical stability of emulsions of sunflower wax in pectin aqueous solutions, in order to evaluate their potential use for the production of edible films.

## **2. Materials and methods**

### **2.1. Materials**

Sunflower waxes were extracted and recovered from two waste samples of “filter cakes” generated in the winterization of sunflower oil (normal and high-oleic hybrids). Each waste material was first fractionated using hot n-hexane as solvent, and then the hot solution was filtered to remove the insoluble particles obtaining a solution containing oil and waxes. Then the waxes were recovered from the oil by successive washings with cold n-hexane according to the procedure described by Chalapud et al. (Chalapud, Baümler, & Carelli, 2016), obtaining the wax samples SFW1 from the normal hybrid and SFW2 from the high-oleic hybrid. Wax samples SFW1 and SFW2, which are in solid state at room temperature (melting point = 76.70 and 80.53 °C, respectively), were characterized in a previous work (Chalapud et al., 2016). Low methoxyl citrus pectin (GENU PECTIN type LM 104 AS) and the sunflower waxes (SFW1 and SFW2) were used for the preparation of the emulsions NE (with SFW1) and HOE (with SFW2).

### **2.2. Preparation of emulsions**

The emulsions were prepared by hydrating the low-methoxyl pectin with distilled water in three concentrations: 1, 2 and 3% w/w. These mixtures were heated at 85 °C

(temperature above the melting point of the wax) in a water bath until the pectin was completely dissolved, and then the temperature was maintained at 85 °C until the solid waxes were added. The waxes were added in three proportions: 0.1, 0.2 and 0.3 g/g pectin. All samples were homogenized for 10 min using a Pro 200 homogenizer (Pro Oxford Scientific Inc.) at 12,000 rpm, maintaining the temperature at 85 °C to avoid wax crystallization. The resulting emulsions were kept at room temperature and analyzed in the same day. Control samples without waxes were prepared following the same procedure and used for rheological comparison. Three replicates were prepared for each condition.

### 2.3. Rheology

The viscosity of the emulsions was determined using a controlled-stress dynamic rheometer (Anton Paar Physica MCR 301) with a concentric cylinder geometry (rotor diameter = 26.658 mm, rotor height = 40.000 mm, cup diameter = 28.920 mm, volume sample = 13 mL). The tests were performed at 25 °C in ascending manner without pre-shear, and the results were submitted to regression analysis in order to verify the applicability of the rheological model. The range of shear rate ( $\dot{\gamma}$ ) was between 0.1 and 100 s<sup>-1</sup> (Akhtar, Stenzel, Murray, & Dickinson, 2005; Huang, Kakuda, & Cui, 2001). These values were determined to ensure that the experiences were conducted in the region of linear behavior.

The experimental flow curves (shear stress vs. shear rate) were compared to the power law model, which is the typical equation to characterize shear-thinning fluids:  $\tau = K \dot{\gamma}^n$ , where  $\tau$  is the shear stress (Pa),  $K$  is the consistency index (Pa s<sup>n</sup>),  $\dot{\gamma}$  is the shear rate (s<sup>-1</sup>), and  $n$  is the flow index,  $n < 1$  for a shear-thinning fluid and  $n = 1$  for a Newtonian fluid.

### 2.4. Particle Size Distribution

The mean particle size of the emulsions was obtained through a laser diffraction analyzer (HORIBA LA-950 V2). The mean size was expressed in terms of the mean diameter over volume ( $D_{4,3}$ ) and its corresponding standard deviation (SD). The distribution amplitude or dispersion index (Span =  $S$ ) was used to have a measure of the dispersion of the data around the mean size. This parameter was calculated as follows (Palazolo, Sorgentini, & Wagner, 2004):  $S = (d(v.0.9) - d(v.0.1))/d(v.0.5)$ , where  $d(v.0.9)$  and  $d(v.0.1)$  are the 90 and 10% percentiles of the particle size distribution as a volume equivalent sphere diameter, and  $d(v.0.5)$  is the 50% volume percentile, also known as median of the distribution.

### 2.5. Confocal Scanning Laser Microscopy

The samples were observed using a confocal scanning laser microscope (Leica Model TCS SP2) configured with an inverted DM IRE2 microscope, with a He/Ne laser at 543 nm, HCX PL APO 63x 1.2 w CORR objective lens and 400 Hz scanning speed. 2D images were obtained with a resolution of 1024 x 1024 pixels using Leica Confocal software (LCS, Leica Microsystems). Nile Red marker was incorporated to the samples in order to facilitate the observation of lipid substances.

### 2.6. Physicochemical Stability and Destabilization Kinetics

After preparation, approximately 7 mL of each emulsion were dispensed into glass tubes at room temperature in order to monitor their stability over time. Three independent determinations were performed using a TurbiScan MA 2000 analyzer (Formulation, Toulouse, France). The equipment consists of a reading head, which moves from top to bottom along a flat-bottomed cylindrical glass cell containing the sample to be analyzed. This head includes a pulsed near-infrared light source (wavelength = 850 nm) and two synchronous detectors, the transmission and backscattering (BS) detectors. Since the emulsions were opaque, only the BS detector was used for data collection. The BS detector received the light backscattered by the sample at 135° from the incident beam. The head scanned the whole sample height (approximately 70 mm), acquiring BS data every 40  $\mu\text{m}$ . The parameters were represented by a curve showing the percentage of BS light (%BS) as a function of the sample height in mm (stability profiles). The BS measurements were performed at room temperature and repeated approximately every 15 minutes during the first 2 hours of emulsion preparation, then at longer intervals (45 minutes, 1 hour, once a day) until approximately 42 days (1,008 hours). Thus, a superimposition of sample fingerprints or “stability profile” was obtained, which characterized the stability or instability of the sample at different times. After obtaining the stability profiles of the emulsions, they were divided into three zones as follows: Zone 1: 6-25 mm, corresponding to the sedimentation and clarification phenomena; zone 2: 25-60 mm, corresponding to the coalescence phenomenon; and zone 3: 60-67 mm, corresponding to creaming. The 1-6 mm zone was not studied because it corresponded to the bottom surface of the glass tubes.

Using the results of the stability analyses, the destabilization kinetics could be evaluated. The destabilization kinetics of sedimentation, creaming and clarification were evaluated by measuring the peak thickness (maximum thickness) at 50% of the

height at different times in the studied zone. In contrast, the destabilization kinetics of coalescence was measured considering the change in the mean %BS values as a function of time at the middle zone of the glass tube (Herrera, 2012).

### 2.7. Statistical analysis

Experiments were performed in duplicate or triplicate as necessary. For each group of experiments performed with each SFW (normal or high-oleic hybrids), significant differences were analyzed using two-way ANOVA and Tuckey's test ( $\alpha = 0.05$ ). The factors considered were the wax proportion and pectin content. Statistical analysis was performed using InfoStat statistical analysis software, Version 2011 (Di Rienzo et al., 2011).

## 3. Results and discussion

Wax samples SFW1 and SFW2 correspond to normal and high-oleic hybrids, respectively. Both have between 40 and 60 carbon atoms, but they present significant differences in their main wax components (SFW1 = C44 to C52, SFW2 = C46 to C54) and fatty acid profiles (mainly C18:1 = 20.2 and 42.6%, C18:2 = 22.1 and 5.9% for SFW1 and SFW2, respectively) (Chalapud et al., 2016). The resulting emulsions (NE from SFW1 and HOE from SFW2) were milky and opaque due to the wax presence (Fig. 1b), and this characteristic was more evident with increasing SFW content. The solutions prepared with pectin alone (control) were translucent (Fig 1a).

### 3.1. Rheology

Shear stress (Pa) as a function of shear rate ( $s^{-1}$ ) for aqueous emulsions of pectin and SFW, NE and HOE, are shown in Fig. 2. In all cases, it is possible to observe a linear relationship between the variables. The increase in pectin content produced higher shear stress values, a behavior that is attributed to the thickening properties of this polysaccharide. For the emulsions prepared with 2 and 3% w/w pectin, the increase in wax proportion implied an increase in the shear stress, whereas for emulsions containing 1% w/w pectin, this increase was only evident when the SFW proportion was of 0.3 g/g pectin (Fig. 2a and 2d). This fact suggests that SFW had a smaller contribution on the emulsions with the lowest pectin level (1% w/w), compared to the control emulsions (without added SFW).

For the shear rate range considered, the most appropriate model to explain the rheological behavior of the emulsions was the Ostwald-de Waele or power law model, in which the shear stress is given by  $\tau = K \dot{\gamma}^n$  (where  $\tau$  is the shear stress,  $\dot{\gamma}$  is the



shear rate,  $K$  is the consistency index, and  $n$  is the flow behavior index). The values of  $n$  were close to 1 (0.9969 - 1.0875), indicating that the behavior of all the emulsions was close to Newtonian. The apparent viscosities obtained with the Ostwald-de Waele model are shown in Table 1. The apparent viscosities of the emulsions prepared with waxes from the high-oleic hybrid (HOE) were significantly higher than those of the emulsions prepared with the normal hybrid (NE) ( $p < 0.05$ ), except for those observed for the emulsions with 1% w/w pectin and wax proportions of 0.1 ( $p = 0.4590$ ) and 0.2 ( $p = 0.0555$ ). According to Mohammed et al., (Mohammed, Okoye, & Salisu, 2016), lower viscosity emulsions produce a smaller droplet size distribution (see Table 2), and in turn lower destabilization rates (as it will be shown later). This was more evident for emulsions with 3% w/w pectin, whose viscosities for NE were significantly lower ( $p < 0.05$ ) than those found for HOE.

Using two-way ANOVA, no interaction was found between wax proportion and pectin contents ( $p = 0.3993$ ). In NE, the wax proportion was not significant ( $p = 0.9999$ ); on the other hand, the pectin content ( $p \ll 0.01$ ) was significant, resulting in higher viscosity values with increasing pectin content. In the case of HOE, an interaction between pectin and wax proportion ( $p \ll 0.01$ ) was determined. A higher pectin content and wax proportion led to an increase in the viscosities of HOE ( $p \ll 0.01$ ); however, a small variation of the viscosities in the emulsions with 2% w/w pectin was observed. The overall rheological properties of the emulsions were clearly influenced by the pectin content, with the emulsions being more viscous when the pectin content was increased.

### 3.2. Particle Size Distribution

The distribution of the droplet diameters with respect to volume percent for NE and HOE are shown in Fig. 3 and their diameters over volume ( $D_{4,3}$ ) are summarized in Table 2.

The mean diameters were between 4.79 and 8.61  $\mu\text{m}$  for NE, and 4.31 and 8.02  $\mu\text{m}$  for HOE, with HOE showing generally lowest values and narrowest ranges. Although there is little information in the literature about emulsions using waxes, these results are similar to those reported for W/O emulsions using soybean oil as oil phase and polyglycerol polyricinoleate as surfactant (average weighted diameter of 4  $\mu\text{m}$ ), and lower than those observed for emulsions prepared with low methoxyl pectin (1%, w/w) in the aqueous phase (average weighted diameter between 18.1 and 38.2  $\mu\text{m}$ ) (Li et al., 2012). On the other hand, Martini et al. (Martini, Carelli, & Lee, 2008) obtained

larger crystals (average weighted diameter between 10.5 and 18.2  $\mu\text{m}$ ) when they analyzed the addition of sunflower waxes to anhydrous milk fat.

In both emulsions, the statistical analysis of the particle size revealed an interaction between the two factors, wax proportion and pectin content ( $p \ll 0.01$ ). Analyzing the effect of increasing the pectin content, the size distribution of the emulsions with 1% of pectin and lower wax proportion (NE = 0.2 and 0.1 wax proportion, see Fig. 3a and 3b; HOE = 0.1 wax proportion, see Fig. 3d) had a Gaussian shape with a very small shoulder on the left, suggesting the presence of a second population of lower mean diameter in the emulsions. Emulsions with 1% of pectin and higher wax content exhibited a gradual decline to the left (NE = 0.3 wax proportion, see Fig. 3c; HOE = 0.2 and 0.3 wax proportion, see Fig. 3e and 3f) and an incipient peak in the 100  $\mu\text{m}$  area, indicating droplet aggregation/coalescence. With increasing pectin content, the distributions had narrower and more well-balanced ranges. When the distribution of a system with many small particles and a few big particles is expressed in number, a monomodal graph is obtained. On the other hand, if the distribution of that system is expressed in volume, a bimodal graph is obtained. Big particles are more notorious when the distribution is expressed in volume (Herrera, 2012).

Table 3 presents the Span data or amplitude distributions, showing higher degrees of dispersion for samples with 1% w/w of pectin. For a wide distribution, droplets have more free space to move around, and thus it is easier for the sample to flow, which is in agreement with a lower viscosity, as shown in Table 1.

It has been stated that decreasing particle size increases viscosity (Barnes, 1994), and that was the case for some of the studied emulsions. For NE, the increase in pectin content from 2 to 3% w/w (Table 2, see columns, uppercase letters) resulted in higher viscosity (Table 1, see columns, uppercase letters) and lower values of mean particle diameter (Table 2, see columns, uppercase letters), regardless of the wax proportion. This indicates that a higher pectin content in the emulsions contributed to the stabilization of the particles without particle aggregation. In contrast, HOE formulated with waxes from high-oleic hybrid and a wax proportion of 0.2 g/g pectin had a slight increase in the mean diameter when the pectin content was increased. As for the wax proportion increase (Table 2, see rows, lowercase letters), significant differences were observed between the mean particle size values of NE and HOE samples ( $p \ll 0.01$ ). A decrease in the mean values with SFW addition was evidenced in NE, especially when pectin content increased from 2 to 3% w/w, while in HOE no definite tendency was observed. This revealed differences in the behavior according to the origin of the waxes that evidently arises from their different composition, especially in their

percentage of unsaturated fatty acids. In fact, some authors suggest that the unsaturation of fatty acids causes different interactions between molecules that could affect the interfacial tension, and thus the droplet size (Bai & McClements, 2016; Berton-Carabin, Ropers, & Genot, 2014). Gaonkar et al. (Gaonkar, 1989), measured the interfacial tensions of vegetable oil/water systems, observing less interfacial tensions for purified unsaturated oils (30.7 dyn/cm for soybean oil and 31.0 dyn/cm for olive oil). In the present work, NE are formulated with waxes containing more unsaturated fatty acids (C18:1 = 20.2 %; C18:2 = 22.1) than HOE (C18:1 = 42.6%; C18:2 = 5.9%) (Chalapud et al., 2016), and so a lower interfacial tension would be expected for NE. Thus, NEs would be easier to emulsify, showing a smaller amplitude/span distribution at higher pectin contents (2% and 3% w/w) and wax proportions (0.2 and 0.3 g/g pectin) (Table 3). NE with 3% w/w of pectin content and high wax proportion also showed a smaller mean diameter mean than HOE.

The distribution analysis performed in the present study highlights the important stabilizing role of pectin in the emulsions, influencing the average particle size and its uniformity. The emulsion stabilizing properties of pectin was explained by Leroux et al. (Leroux et al., 2003), who used a model to describe its ability to produce fine and stable emulsions.

### 3.3. Confocal Microscopy

Confocal images of NE and HOE with 3% w/w pectin are shown in Fig. 4. In all the emulsions, it was possible to visualize the SFW, observing a good dispersion and distribution of the SFW particles in the medium, and the formation of aggregates was not detected. This would confirm stabilizing capacity of pectin with respect to the SFW particles, which attain a homogeneous and uniform distribution in the medium. The good dispersion of the lipid component in the matrix makes these emulsions especially adequate to be used in the production of edible films, since this fact has a significant effect on the final barrier properties of the films.

### 3.4. Physicochemical Stability and Destabilization Kinetics

Fig. 5, show representative stability profiles of NE and HOE, exhibiting the coupling of three main destabilization phenomena: sedimentation, coalescence and creaming. It was also possible to analyze the level of clarification, which, although not being a destabilization phenomenon itself, is the result of particle migration.

Sedimentation (observed at the bottom of the tube) was identified mainly in both emulsions (NE and HOE) prepared with 1% w/w of pectin content. This phenomenon

showed positive values of %BS, which decreased with increasing SFW proportion, reaching lower positive %BS values when the SFW proportion was 0.3 g/g pectin, as well as negative values, indicating the gradual clarification in the samples. For both emulsions (NE and HOE) formulated with 0.1 and 0.2 g/g pectin, it was possible to observe that the positive values of %BS increased at different times, with sedimentation taking place after about 24 and 72 hours for NE (Fig. 6a, 6c), while for HOE it started later (144-270 hours) (Fig. 6b, 6d). When the pectin level was 2% w/w and the SFW proportion was 0.1 g/g pectin, the emulsion profiles showed positive %BS values, with those values being higher for NE. For this emulsion, the onset of sedimentation was evidenced approximately 72 hours after preparation, while for HOE this phenomenon was more noticeable after 600 hours (Fig. 6e, 6f). Thus, waxes from the high-oleic hybrid provided more stable emulsions with respect to sedimentation, which is consistent with their smaller mean particle sizes (See Table 2). This follows Stokes' law, according to which sedimentation velocity is inversely proportional to the square of the particle size, and therefore it sediments more slowly.

The most important reason for the reduction in the sedimentation of the emulsions associated with increasing wax proportion has its origin in the closeness between the particles; so that as the larger particles move upward, they collide and stick to the smaller ones, thus producing a movement towards the upper part (creaming) and not towards the lower part (sedimentation) (McClements, 1999).

The influence of the SFW proportion on the sedimentation phenomenon of NE and HOE with 1% w/w of pectin can be observed in its kinetics, which is shown in Fig. 7. Higher destabilization rates were observed in emulsions with wax proportions of 0.1 g/g pectin, with sedimentation being counteracted when the SFW proportion increased. This effect was more noticeable in NE, whose destabilization rate was higher compared to HOE, and therefore the level of reduction of that rate caused by the addition of SFW was larger.

In Fig. 8 the influence of SFW proportion on the clarification kinetics is shown. Emulsions (NE and HOE) with 2% w/w pectin and proportions of 0.2 and 0.3 g/g pectin (Fig. 8a) exhibited similar behaviors, with their destabilization rate decreasing at approximately 150 hours (6 days) of measurement, while for the emulsions (NE and HOE) with 3% w/w pectin (Fig. 8b) it decreased at approximately 600 hours (25 days) of measurement.

In the stability profiles of NE and HOE emulsions (Fig. 5) it was possible to observe negative %BS values in the middle zone of the samples. This behavior was induced by

a gradual particle size increase, characteristic of coalescence phenomena, which was more pronounced as the pectin content and wax proportion increased.

The comparative kinetics of the coalescence process (Zone 2) is presented in Fig. 9. The behavior of both emulsions NE and HOE was similar independently of the wax origin. This process was promoted by the increase in SFW and pectin content, giving smaller backscattering values. But despite the higher pectin content and the differences between the interfacial tensions due to the different fatty acid composition of the waxes, the interfacial resistance at the surface of the droplets was not sufficient to prevent their union and thus the evolution of the coalescence phenomenon. These results are in agreement with those reported by Kermani et al., who observed the formation of large oil droplets during the storage of O/W emulsions prepared with apple pectin, which was attributed to low interfacial resistance (Kermani, Shpigelman, Pham, Van Loey, & Hendrickx, 2015). At all pectin levels, destabilization rates were the highest for the emulsions with a wax proportion of 0.3 g/g pectin (Fig. 9). The kinetic destabilization rate was slower after approximately one third of the measurement time (400 hours) in the emulsions with 1 and 2% w/w pectin (Fig. 9a, 9b, 9d and 9e). The opposite behavior was observed in the emulsions prepared with 3% w/w pectin (Fig. 9c and 9f), where the coalescence rate increased after approximately 400 hours of measurement (greater slope in the kinetic curve). The coalescence process in emulsions is determined by the breaking of the interfacial membrane that surrounds the particles, generating the union of those particles after their collision (Rousseau, 2000; Walstra, 1996). If the continuous phase layer surrounding the particles is thin and weak, the probability that coalescence occurs is high. Therefore, the increase in the coalescence destabilization rates for the emulsions with the highest SFW proportion may be due to the fact that the pectin content was not enough to achieve the complete saturation of the surface particles, and the viscosity increase of the continuous phase did not reduce the particle movement generated over time. Thus, a weak interfacial membrane was obtained that offered little resistance to the particle union. In addition, the extended contact time (1,008 hours) between particles played an important role in the evolution of this process.

An increase in the backscattering signal was observed over a small portion of the sample (10 mm) at the top of the tube (Zone 3), indicating the presence of creaming phenomena (positive peaks %BS). The onset of this phenomenon was between 24 and 48 hours, being more noticeable at longer measurement times. The creaming kinetic destabilization is shown in Fig. 10, where the behavior of emulsions NE and HOE was similar. SFW addition induced an increase in the destabilization rate, decreasing after

200 hours of measurement (8 days). The polydispersion of the emulsions contributes to the existence of the creaming phenomenon because the larger particles tend to migrate more quickly towards the top. However, although particle size distributions (Fig. 3) indicate that there was a greater dispersion when the pectin content was low, the creaming phenomenon found for NE and HOE indicates that this behavior did not depend mainly on the greater or smaller polydispersity, since in all the emulsions (NE and HOE) this process occurred at similar times. These results are in agreement with those reported by other authors who indicated that the improvement of stability against creaming is due mainly to the contribution to the viscosity of the continuous aqueous phase (Guo et al., 2014; Kermani et al., 2015).

#### **4. Conclusions**

The analysis of the physical properties and stability of emulsions is of a great importance for the production of edible films, since they affect the final characteristics of films to provide adequate protection to the food. The rheological behavior was well described by the power law model, being evident an increase in viscosity and shear stress of the emulsions with increasing pectin content and wax proportion. Higher pectin contents produced tighter size distributions. The micrograph analysis revealed the presence of crystal of SFW in the emulsions, with a satisfactory particle dispersion attributed to the emulsifying capacity of pectin, which is important for the subsequent permeability of the film once it has been developed. Despite the increase in the viscosity of the emulsions with increasing pectin and wax proportion, the sedimentation phenomenon in the emulsions was dissipated, but coalescence and creaming became more evident. These three phenomena (sedimentation, coalescence and creaming) were evidenced at long test times independently of the SFW origin, at 24 hours or more after emulsion preparation. The emulsions with waxes from high-oleic sunflower exhibited a smaller particle size when prepared with 1 and 2% of pectin, while the emulsions with SFW from a normal hybrid and 3 % pectin presented lower viscosity and produced a smaller droplet size distribution. The stability found for the studied emulsions shows their potential use for the manufacture of edible films.

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**Conflict Of Interest Statement**

The authors have declared no conflict of interest

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**Table 1.** Apparent viscosities (Pa.s) based on the power model for pectin and sunflower waxes aqueous emulsions NE and HOE.

	Pectin (% w/w)	Wax proportion (g /g pectin)		
		0.1	0.2	0.3
NE	1	<sup>A</sup> 0.0070 ± 0.0002 <sup>a, A</sup>	<sup>A</sup> 0.0077 ± 0.0006 <sup>a, A</sup>	<sup>A</sup> 0.0080 ± 0.0006 <sup>a, A</sup>
	2	<sup>A</sup> 0.0231 ± 0.0011 <sup>a, B</sup>	<sup>A</sup> 0.0244 ± 0.0010 <sup>b, B</sup>	<sup>A</sup> 0.0230 ± 0.0019 <sup>a, B</sup>
	3	<sup>A</sup> 0.0587 ± 0.0004 <sup>a, C</sup>	<sup>A</sup> 0.0575 ± 0.0001 <sup>a, C</sup>	<sup>A</sup> 0.0577 ± 0.0004 <sup>a, C</sup>
HOE	1	<sup>A</sup> 0.0076 ± 0.0007 <sup>a, A</sup>	<sup>A</sup> 0.0086 ± 0.0000 <sup>ab, A</sup>	<sup>B</sup> 0.0098 ± 0.0003 <sup>b, A</sup>
	2	<sup>B</sup> 0.0301 ± 0.0009 <sup>b, B</sup>	<sup>B</sup> 0.0296 ± 0.0003 <sup>b, B</sup>	<sup>B</sup> 0.0325 ± 0.0002 <sup>a, B</sup>
	3	<sup>B</sup> 0.0731 ± 0.0002 <sup>a, C</sup>	<sup>B</sup> 0.0773 ± 0.0020 <sup>b, C</sup>	<sup>B</sup> 0.0777 ± 0.0002 <sup>b, C</sup>

Mean values ± standard deviation. n = 2. Values in the same row followed by different lowercase letters (waxes effect addition) are significantly different (p < 0.05) by the Tukey Test. Values in the same column followed by different uppercase letters (pectin effect addition) are significantly different (p < 0.05) by the Tukey Test. The values in the same column preceded by different uppercase letters (comparison of emulsions in each concentration of pectin and wax proportion) are significantly different (p < 0.05) by the Tukey Test.

**Table 2.** Diameter over volume,  $D_{4,3}$  ( $\mu\text{m}$ ) and standard deviation ( $\mu\text{m}$ ) for pectin and sunflower waxes aqueous emulsions NE and HOE.

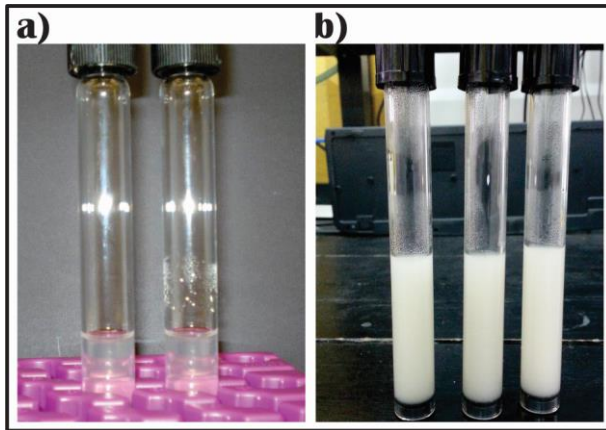
	Pectin (% w/w)	Wax proportion (g /g pectin)		
		0.1	0.2	0.3
NE	1	8.16 $\pm$ 0.78 <sup>a, B</sup>	8.46 $\pm$ 0.19 <sup>a, C</sup>	8.61 $\pm$ 0.29 <sup>a, C</sup>
	2	7.62 $\pm$ 0.09 <sup>c, B</sup>	6.44 $\pm$ 0.01 <sup>b, B</sup>	6.21 $\pm$ 0.02 <sup>a, B</sup>
	3	5.31 $\pm$ 0.04 <sup>c, A</sup>	5.09 $\pm$ 0.07 <sup>b, A</sup>	4.79 $\pm$ 0.04 <sup>a, A</sup>
HOE	1	6.21 $\pm$ 0.13 <sup>c, B</sup>	4.55 $\pm$ 0.06 <sup>b, A</sup>	4.31 $\pm$ 0.10 <sup>a, A</sup>
	2	6.09 $\pm$ 0.09 <sup>b, B</sup>	5.71 $\pm$ 0.11 <sup>a, B</sup>	5.97 $\pm$ 0.09 <sup>b, B</sup>
	3	5.38 $\pm$ 0.03 <sup>a, A</sup>	6.48 $\pm$ 0.51 <sup>b, C</sup>	8.02 $\pm$ 0.19 <sup>c, C</sup>

Mean values  $\pm$  standard deviation. n = 3. Values in the same row followed by different lowercase letters (waxes effect addition) are significantly different ( $p < 0.05$ ) by the Tukey Test. Values in the same column followed by different uppercase letters (pectin effect addition) are significantly different ( $p < 0.05$ ) by the Tukey Test.

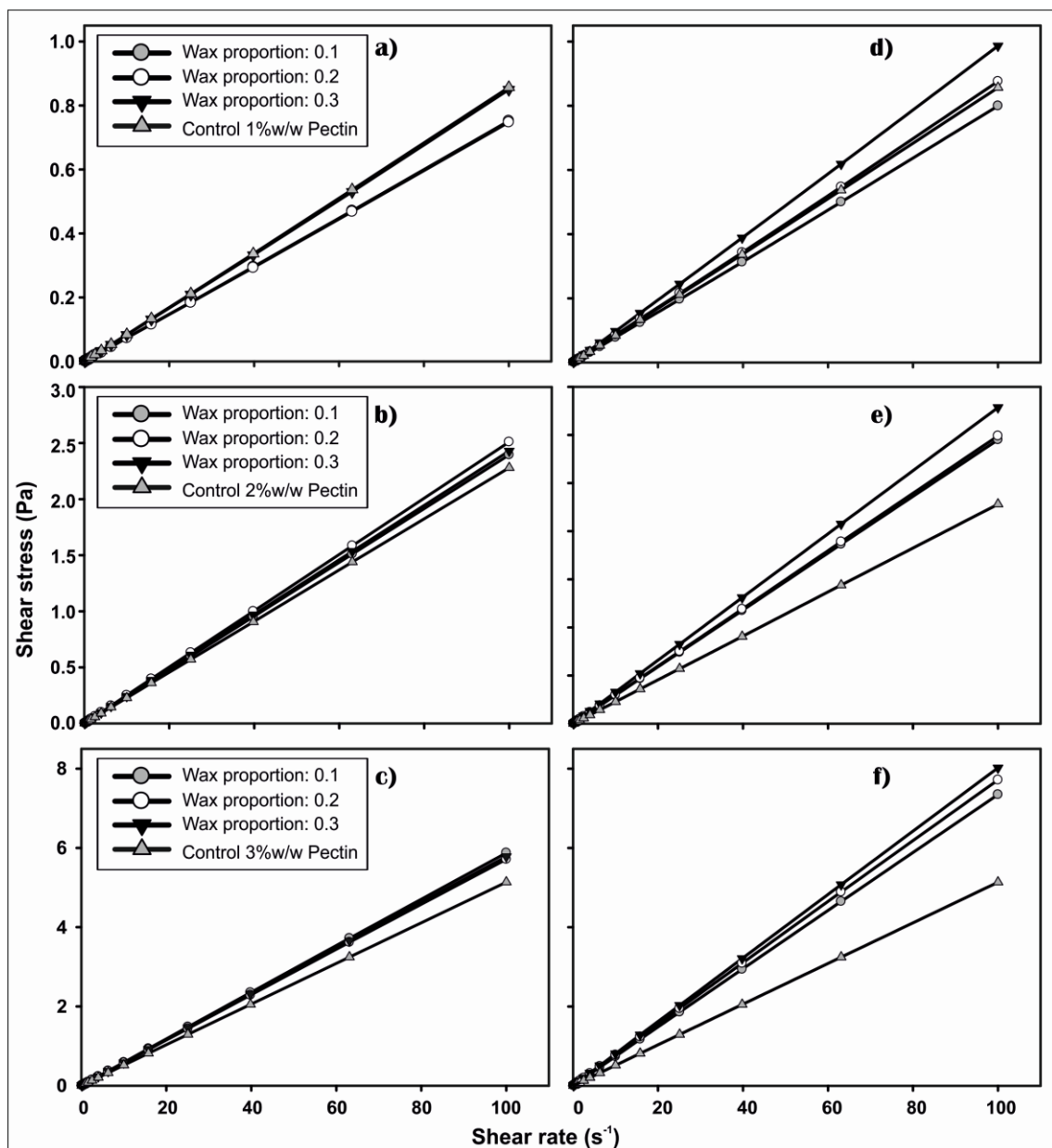
**Table 3.** Span data (S) o amplitude distributions of particle size for pectin and sunflower waxes aqueous emulsions NE and HOE.

	Pectin (% w/w)	Wax proportion (g /g pectin)		
		0.1	0.2	0.3
NE	1	<sup>A</sup> 1.98 ± 0.08 <sup>ab, B</sup>	<sup>A</sup> 1.86 ± 0.03 <sup>a, C</sup>	<sup>B</sup> 2.04 ± 0.05 <sup>b, C</sup>
	2	<sup>A</sup> 1.30 ± 0.01 <sup>a, A</sup>	<sup>A</sup> 1.41 ± 0.01 <sup>b, B</sup>	<sup>A</sup> 1.32 ± 0.01 <sup>a, B</sup>
	3	<sup>A</sup> 1.22 ± 0.01 <sup>b, A</sup>	<sup>A</sup> 1.10 ± 0.02 <sup>a, A</sup>	<sup>A</sup> 1.12 ± 0.01 <sup>a, A</sup>
HOE	1	<sup>A</sup> 1.97 ± 0.07 <sup>c, C</sup>	<sup>A</sup> 1.85 ± 0.08 <sup>b, C</sup>	<sup>A</sup> 1.63 ± 0.02 <sup>a, C</sup>
	2	<sup>B</sup> 1.68 ± 0.06 <sup>c, B</sup>	<sup>B</sup> 1.54 ± 0.03 <sup>b, B</sup>	<sup>B</sup> 1.48 ± 0.04 <sup>a, B</sup>
	3	<sup>B</sup> 1.29 ± 0.01 <sup>a, A</sup>	<sup>B</sup> 1.30 ± 0.01 <sup>ab, A</sup>	<sup>B</sup> 1.31 ± 0.01 <sup>b, A</sup>

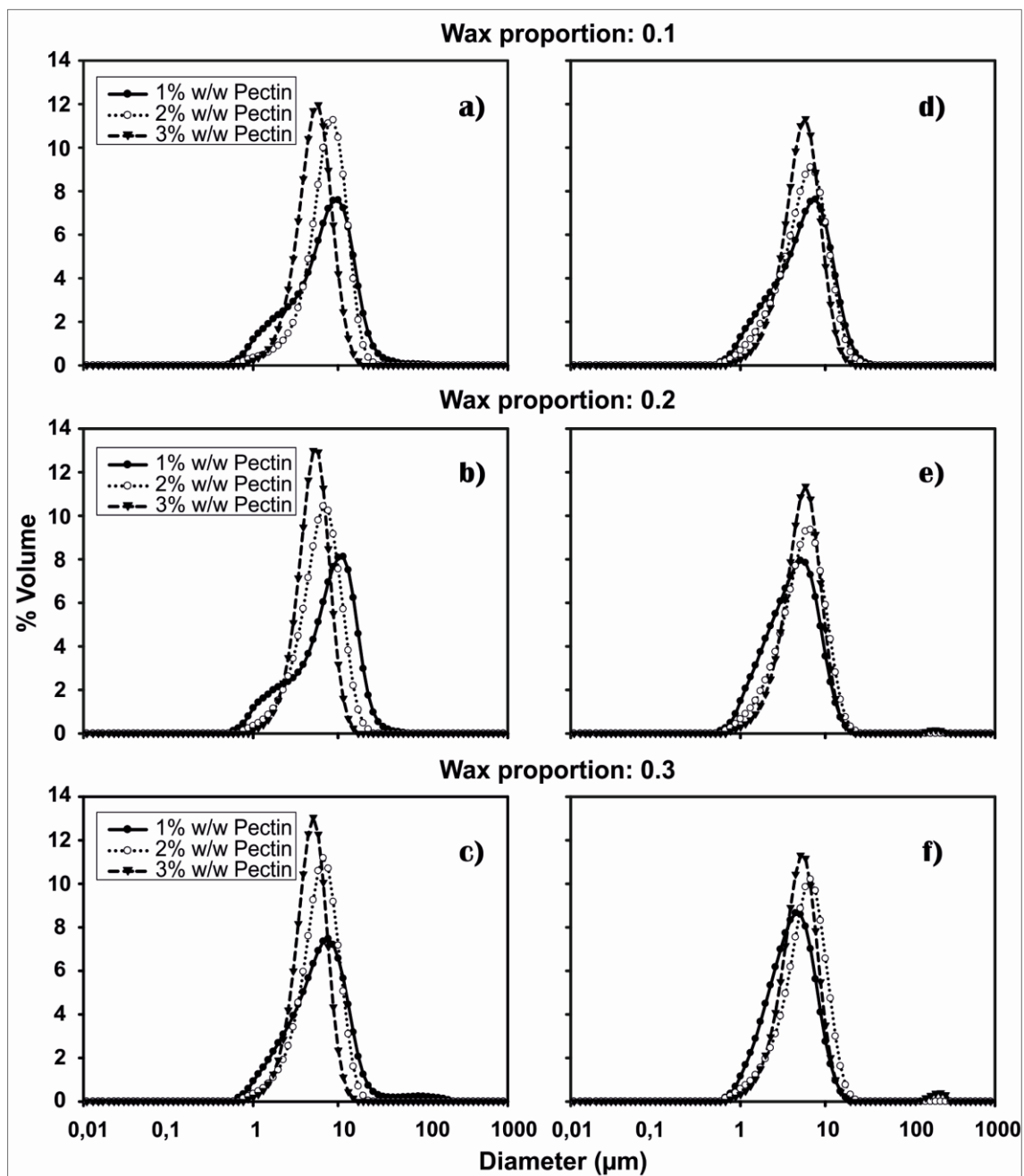
Mean values ± standard deviation. n = 3. Values in the same row followed by different lowercase letters (waxes effect addition) are significantly different (p < 0.05) by the Tukey Test. Values in the same column followed by different uppercase letters (pectin effect addition) are significantly different (p < 0.05) by the Tukey Test. The values in the same column preceded by different uppercase letters (comparison of emulsions in each concentration of pectin and wax proportion) are significantly different (p < 0.05) by the Tukey Test.



**Fig. 1** Photographs of the emulsions placed on tubes (control samples: a; SFW-pectin emulsions: b).

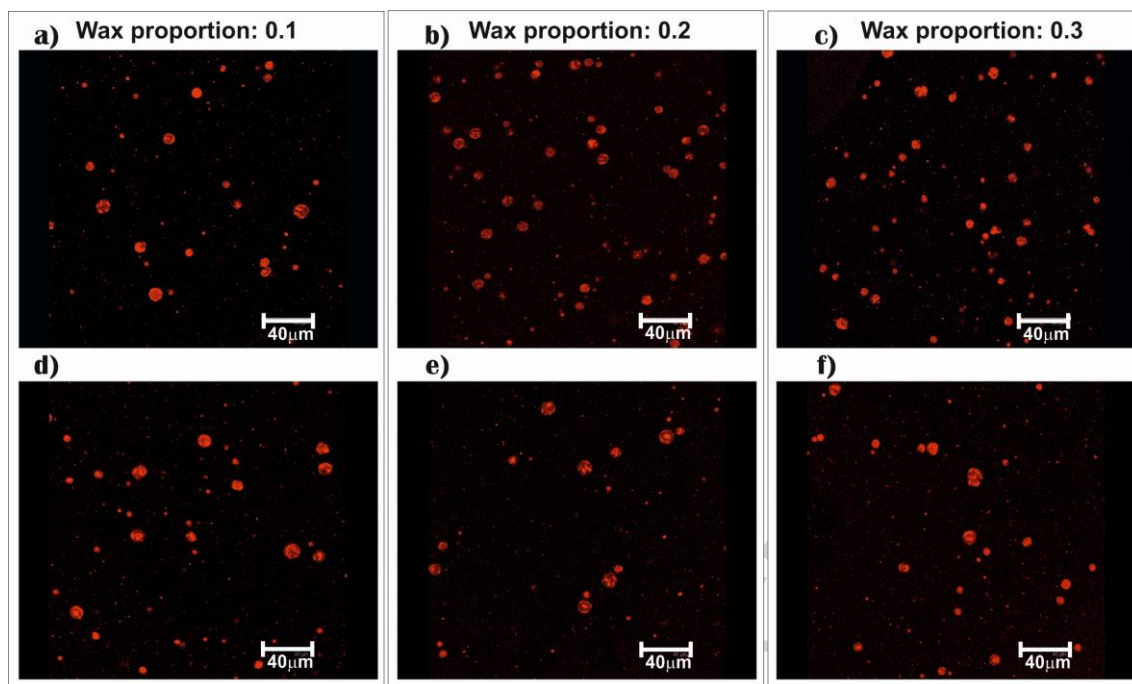


**Fig. 2** Rheological behavior of the emulsions (NE: a, b, c; HOE: d, e, f) with different pectin contents (1% w/w pectin: a and d; 2% w/w pectin: b and e; 3% w/w pectin: c and f) and wax to pectin proportions.

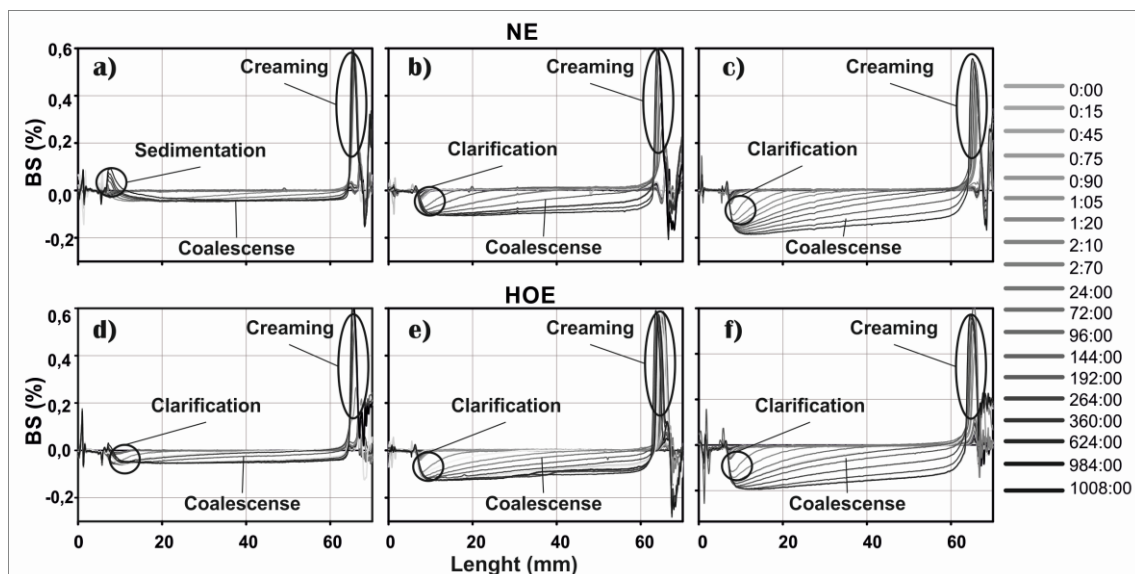


**Fig. 3** Particle size distribution with different wax to pectin proportions. NE: a, b, c. HOE: d, e, f.

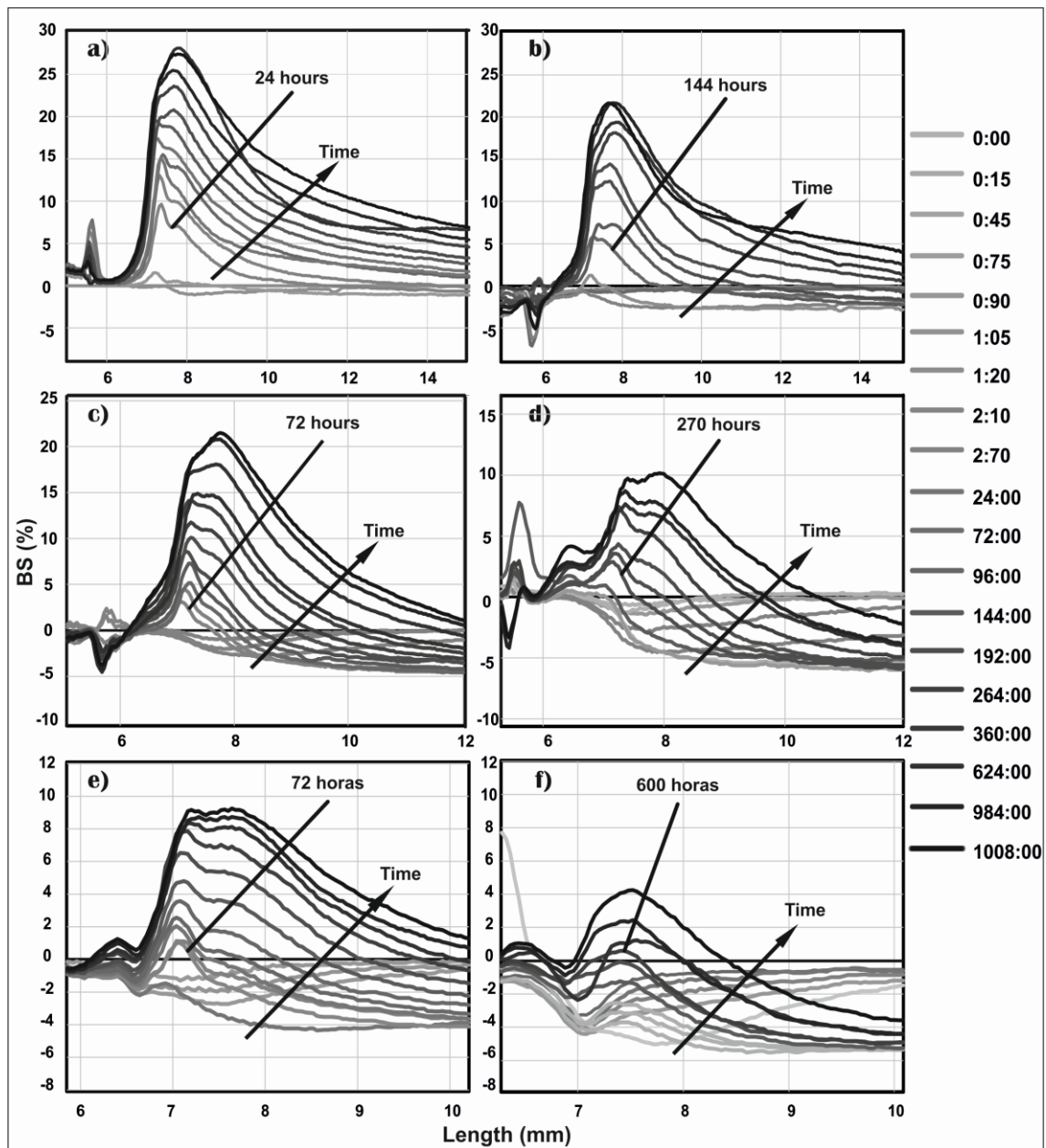




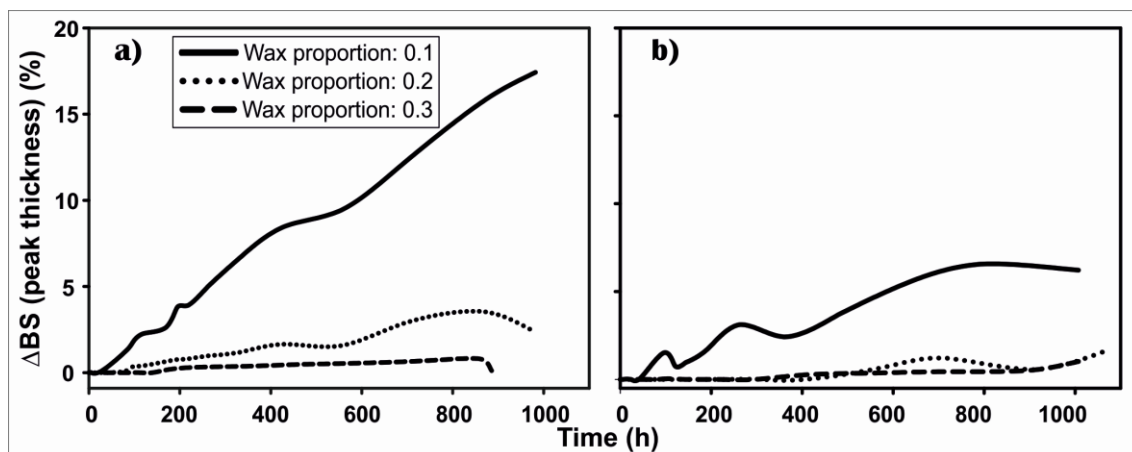
**Fig. 4** Confocal laser microscopy images (3% w/w pectin) with different wax to pectin proportions. NE: a, b, c. HOE: d, e, f.



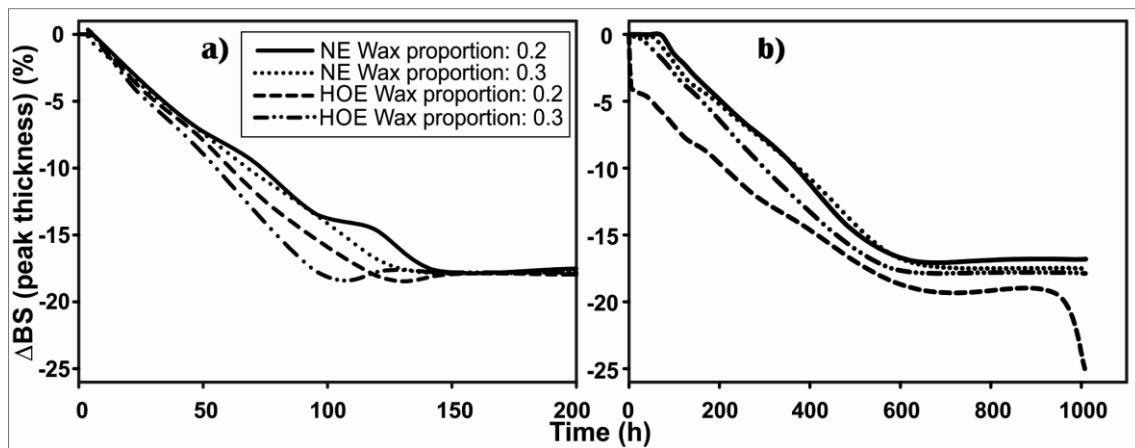
**Fig. 5** Representative delta backscattering profiles for NE and HOE (NE: a, b, c; HOE: d, e, f). Wax proportions: 0.1 (a, d), 0.2 (b, e), 0.3 (c, f).



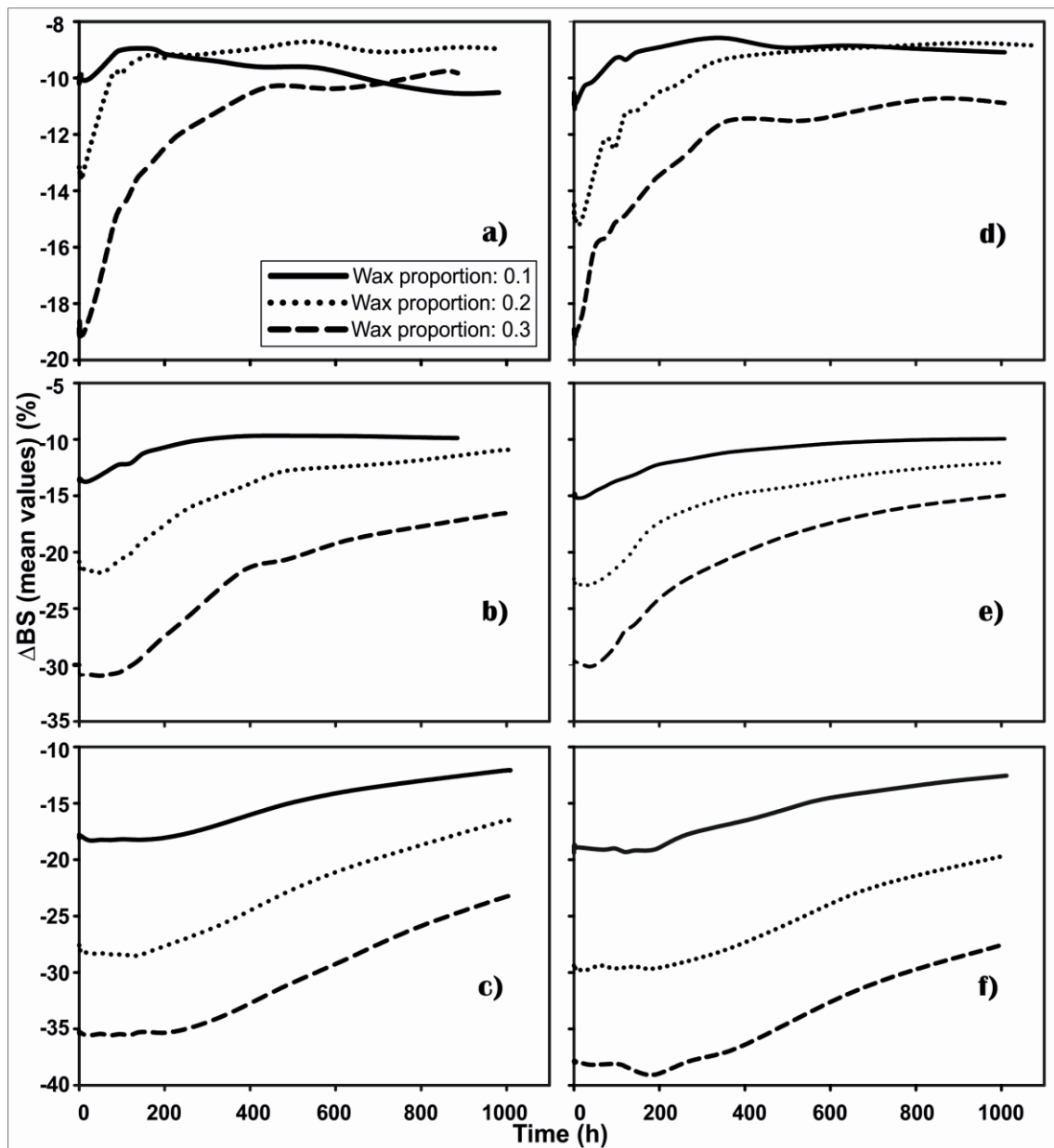
**Fig. 6** Sedimentation zone for emulsions with 1% w/w pectin: NE (a, c) and HOE (b, d), wax proportion: 0.1 (a, b), 0.2 (c, d). Sedimentation zone for emulsions with 2% w/w pectin: NE (e) and HOE (f) , wax proportion: 0.1.



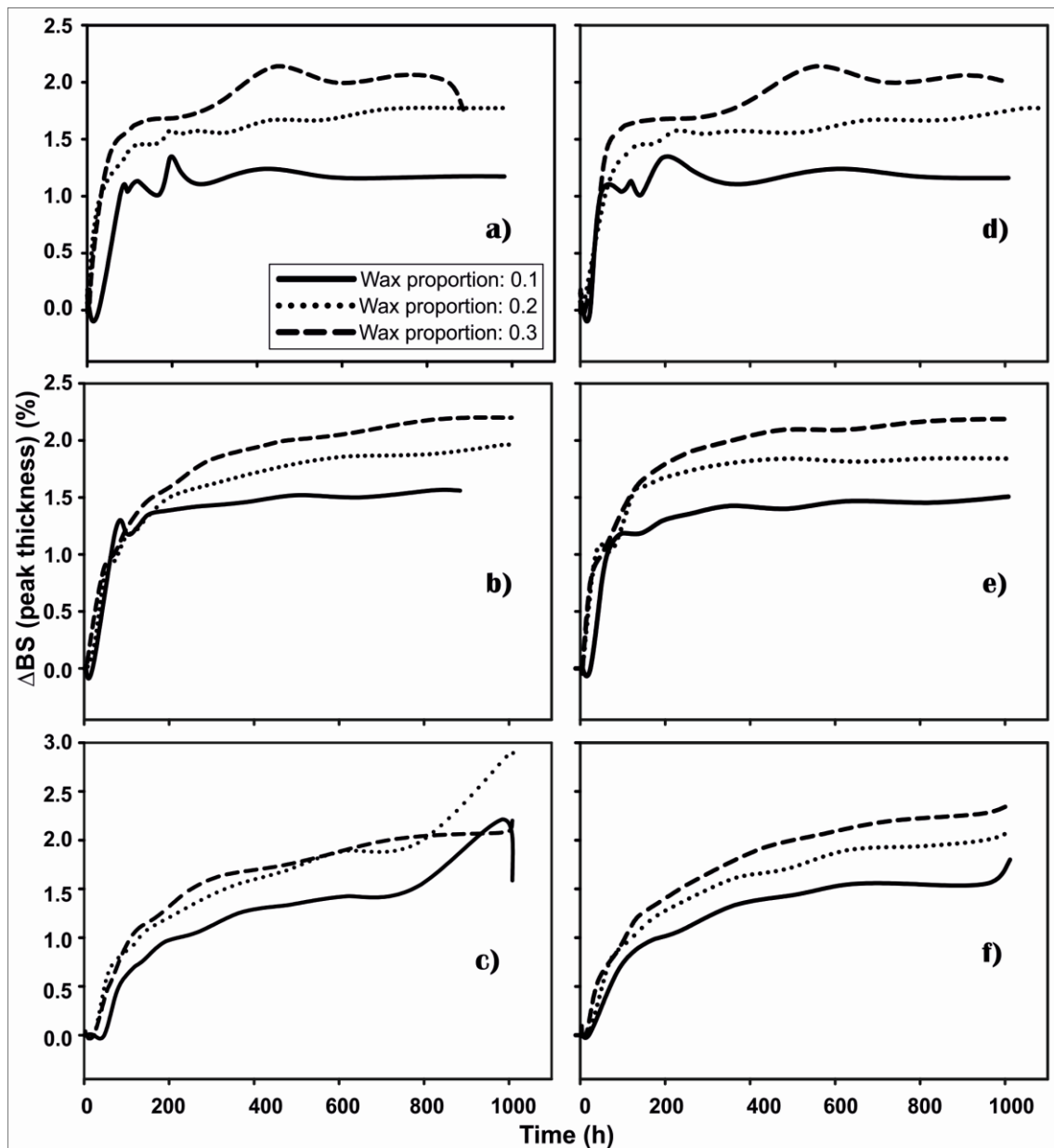
**Fig. 7** Destabilization kinetics. Sedimentation (Zone 1) for NE (a) and HOE (b) with 1% w/w pectin.



**Fig. 8** Destabilization kinetics. Clarification (Zone 1). Effect of sunflower wax content (wax proportions: 0.2 and 0.3) with different pectin contents (a: 2% w/w pectin, b: 3% w/w pectin).

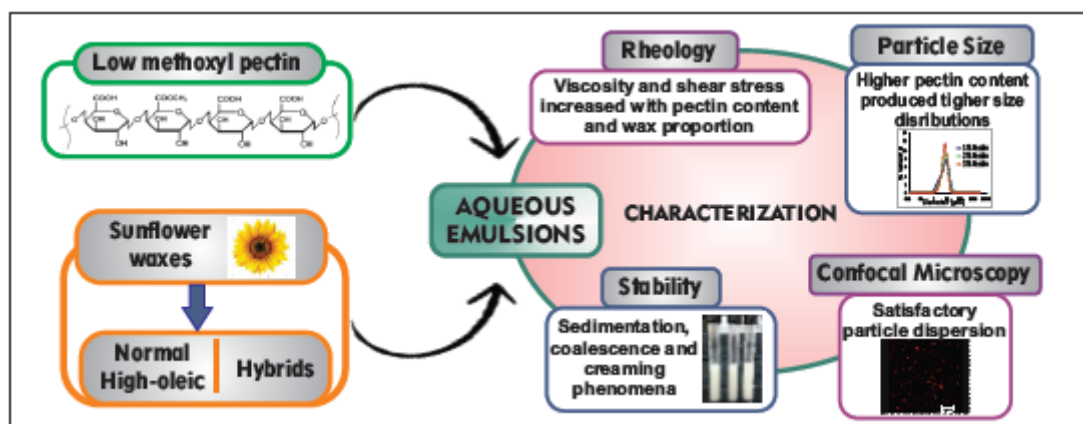


**Fig. 9** Destabilization kinetics. Coalescence (Zone 2) for NE (a, b and c) and HOE (d, e and f). Effect of wax proportion and pectin content (1% w/w pectin: a and d; 2% w/w pectin: b and e; 3% w/w pectin: c and f).



**Fig. 10** Destabilization kinetics. Creaming (Zone 3) for NE (a, b and c) and HOE (d, e and f). Effect of wax proportion and pectin content (1% w/w pectin: a and d; 2% w/w pectin: b and e; 3% w/w pectin: c and f).

Graphical abstract





## Highlight

- Low-methoxyl pectin and sunflower wax aqueous emulsions were prepared
- The physical properties, particle size and stability were studied
- Destabilization phenomena were observed at long test times
- The production of edible films with pectin and sunflower wax emulsions is promising

ACCEPTED MANUSCRIPT