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Physical Properties of Aqueous Solutions of Pectin Containing Sunflower Wax

Erica R. Baumler · Amalia A. Carelli ·
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Abstract The aim of this study is to investigate the physical properties of aqueous solutions of pectin (PA) containing sunflower wax (SFW), which are used as a basis for producing edible films. The stability and the rheological and microstructural characteristics of SFW/PA mixtures were evaluated. SFW/PA mixtures formed oil-in-water emulsions that were milky and opaque in appearance and were stable towards phase separation. Polarized micrographs revealed the presence of wax crystals, whose size decreased as pectin concentration increased. The rheological behavior of the aqueous solutions of pectin containing different amounts of SFW were best described by the generalized power law model of Herschel–Bulkley (H–B), which gave the best fit in all the range of shear rate values. Apparent viscosities and yield stress were determined using this model, and both properties increased with increasing pectin content. The apparent viscosity values were between 0.0095 and 0.1031 Pa s. SFW addition resulted in a small decrease in viscosity for emulsions formulated with 1 and 2 % PA, but the opposite effect was observed for emulsions formulated with 3 % PA. In addition, shear stress values were higher for emulsions with higher PA content, but were not affected by SFW addition.

Keywords Sunflower waxes · Pectin · Edible film · Emulsion

Introduction

Pectins are one of the polysaccharides most commonly used in edible film formulation; their main disadvantage is the hydrophilic capacity of their macromolecules [1], which renders them inefficient as a barrier against water and water vapors. Waxes are the lipid that is usually used to improve the barrier properties against water and water vapor of edible film. They can be found in nature in fruits and seeds, being formidable barriers through which permeants diffuse with extremely low mobility [2]. They also represent a significant by-product of the refining process of several vegetable oils.

In recent years, there has been a widespread growing interest and research in the development of edible films as an alternative to synthetic packaging to prevent moisture migration between foods and environment. These films can be produced either as bilayer films, where lipids form a layer over a hydrocolloid matrix, or as emulsified films where the lipid is dispersed in the aqueous matrix.

The formation of a film with a uniform surface is critical to ensure optimum water and water vapor barrier properties. The achievement of this uniform surface strongly depends on the characteristic of the starting solution. That is, it is difficult to obtain a uniform surface when using heterogenous emulsified films. Some experiments have been performed with refined lipid materials that are not miscible in aqueous film-forming solutions and therefore result in low quality films with a non-uniform surface [3]. Crude oils are “impure” systems that contain some amphiphilic minor compounds that can improve their

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miscibility in aqueous solutions. These materials could therefore be used to improve the miscibility of the lipid and aqueous phases and therefore form uniform composite films [4, 5]. Sunflower waxes, which usually contain traces of crude oil, could be used as an additive to facilitate emulsion formation and to form uniform films and improve the water vapor barrier properties of edible films.

Pectins are common materials used to form edible films. These macromolecules are also known to contain some emulsifying properties. The use of pectin as an emulsifying agent in various applications such as flavored, mineral and vegetable oil emulsions and mayonnaise was suggested as early as 1927 [6]. Recently, Kertesz [7] mentioned its emulsifying properties in an extensive review, and Parker et al. [8] reported the ability of pectin to stabilize dairy protein under acidic conditions. More recently, Leroux et al. [9] showed that pectin is able to reduce the interfacial tension between an oil phase and a water phase, producing fine and stable emulsions in the same manner as gum arabic, but at much lower dosage. The authors suggested that the observed emulsifying properties of pectin are most probably due to the protein residues present within the pectin.

Since many edible film formulations are complex mixtures of biopolymers and other components, knowledge of their rheological behavior and thermophysical properties would be useful in optimizing industrial processes [10–12].

Previous work carried out in pectin/waxes solutions reported the rheological characteristics, refractive indices, and the number and average size of diffracting particles per unit volume of the emulsion as a function of wax source [13]. The authors concluded that all the investigated waxes can be used to obtain edible films; however, they did not evaluate the mixture composition and the effect of the pectin/wax ratio on the emulsion and/or film properties.

The aim of this study was to investigate the physical properties of aqueous solutions of pectin containing sunflower oil wax (SFW/PA) in order to test the potential use of sunflower waxes in the production of edible films. Stability as well as rheological and microstructural characteristics of SFW/PA with different pectin/wax ratios were evaluated.

Experimental Procedures

Sample Preparation

A low methoxyl citric pectin (LM 104) AS from GENU PECTIN (Denmark) was used as the film-forming substance. This pectin is cross-linkable with divalent cations such as Ca^{2+} and Mg^{2+} . A filter cake obtained from the dewaxing process of crude sunflower oil only pretreated

with a water degumming was provided by a local factory. This material, consisting of waxes, oil, and filter aid, was used to obtain purified sunflower waxes (SFW). The purification procedure consisted of washing with n-hexane at 50 °C for 30 min, and then filtering the hot solution to eliminate insoluble particles. After this process, a mixture mainly of waxes and oil was obtained. The waxes were then purified by successive washings with cold hexane.

Emulsions were prepared by hydrating the pectin at room temperature and dissolving it in distilled water using a water bath at 80 °C for 15 min. The concentrations used, expressed as weight percent of pectin per aqueous solution of pectin (PA), were 1, 2, or 3 % wt/wt. The necessary amount of SFW (0, 0.2 or 0.4 % wt/PA weight) was added and homogenized through a high-intensity ultrasound generator (HIU, Misonix S-3000; Qsonica, Newtown, CT, USA); an 1/8-in-diameter (3.18-mm) tip (amplitude of the tip = 12 μm) was used for 5 min. During the emulsification process, the samples were maintained inside the water bath at 80 °C to avoid wax crystallization.

Wax Analysis

GC Analysis

The following wax standards of almost 99 % purity (Sigma Chemica, St. Louis, MO, USA) were used for qualitative identification: C32 = lauric acid arachidyl ester ($\text{C}_{32}\text{H}_{64}\text{O}_2$), C36 = stearic acid stearyl ester ($\text{C}_{36}\text{H}_{72}\text{O}_2$), C38 = arachidic acid oleoyl ester ($\text{C}_{38}\text{H}_{74}\text{O}_2$), C40 = arachidic acid arachidyl ester ($\text{C}_{40}\text{H}_{80}\text{O}_2$), C42 = arachidic acid behenyl ester ($\text{C}_{42}\text{H}_{84}\text{O}_2$), and C44 = behenic acid behenyl ester ($\text{C}_{44}\text{H}_{88}\text{O}_2$). The C32 standard was also used as internal standard.

The wax composition of purified SFW was performed by directly dissolving 2 mg of the material in chromatographic grade n-heptane (3 mL), adding 1 mL of standard internal solution (0.02 % C32 in n-hexane), then filtering through a 0.5- μm filter for organic solvents, and finally analyzing by capillary GLC [14]. A Perkin Elmer Auto-System XL gas chromatograph equipped with a FID detector, a programmed-temperature on-column injector and a TotalChrom Workstation v.6.3.1 data processor (Perkin Elmer, MA, USA) was used. The capillary column was an HP-5 (5 % diphenyl and 95 % dimethyl-polysiloxane), fused-silica, 15 m length \times 0.32 mm i.d., 0.25 μm film thickness (Hewlett-Packard, Palo Alto, CA, USA). The operating conditions were: hydrogen at 3 mL/min as carrier gas; oven temperature programming: initial temperature of 80 °C, increasing at 30 °C/min to 200 °C, holding for 1 min, increasing at 3 °C/min to 340 °C, holding for 20 min; on-column injector programmed from

80 °C to 320 °C at 20 °C/min, and injection volume of 2 μ L; FID at 350 °C.

DSC Analysis

The thermo-calorific physical characteristics of purified SFW were determined by means of a DSC 2910 differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE, USA). Approximately 5–10 mg of SFW were placed on an aluminum DSC pan and hermetically sealed. Samples were placed in the DSC chamber and held at –20 °C for 30 min. After this step, samples were heated at 5 °C/min to 120 °C to evaluate their melting profile. Onset temperature (T_{on}), peak temperature (T_p) and enthalpy (ΔH) values were used to characterize the melting behavior of the waxes.

Emulsion Analysis

Stability

The physicochemical stability of the SFW/PA emulsions was studied using a vertical scan macroscopic analyzer (TurbiScan MA 2000, l'Union, France). The TurbiScan analyzer has a reading head, consisting of a pulsed near-infrared light source and two synchronous detectors, which move along a flat-bottomed cylindrical cell while scanning the whole sample height. Since emulsions were opaque, only the backscattering (BS) detector was used for data recompilation. The BS detector received the light backscattered by the product (135°), and the data were acquired by the reading head every 40 μ m. The parameters were represented by a curve showing the percentage of BS light as a function of the sample height in mm. The data acquisition along the product was then repeated, obtaining a superimposition of sample fingerprints, which characterized the stability or instability of the sample (e.g., the more identical the readings, the more stable the system). The destabilization peak found at the bottom of the tube was used to quantify the destabilization kinetics. The change in the width of the destabilization peak at half its height was used to quantify the destabilization kinetics of the samples [15, 16]. Details regarding the use of TurbiScan can be found in several previously published researches [16–19].

After forming the emulsions, approximately 6 mL of each sample were dispensed into glass tubes. The BS measurements were performed at room temperature at different times.

Microscopy

A drop of the emulsions at 80 °C was placed between a slide and a cover-slide, pressed gently and cooled to room

Table 1 Wax distribution in purified SFW

Wax carbon number	Wt %	Wax carbon number	Wt %
40	0.13 \pm 0.06	49	1.32 \pm 0.05
41	0.06 \pm 0.01	50	9.12 \pm 0.31
42	4.02 \pm 0.19	51	0.97 \pm 0.16
43	0.89 \pm 0.04	52	5.92 \pm 0.76
44	22.11 \pm 1.20	53	0.60 \pm 0.16
45	2.60 \pm 0.05	54	3.22 \pm 0.71
46	27.16 \pm 1.24	55	0.27 \pm 0.10
47	2.09 \pm 0.07	56	2.34 \pm 0.54
48	17.18 \pm 0.47		
Total wax content (mg/kg)		754,229 \pm 116,349	

Values are mean of two independent samples analyzed by duplicate \pm standard deviation

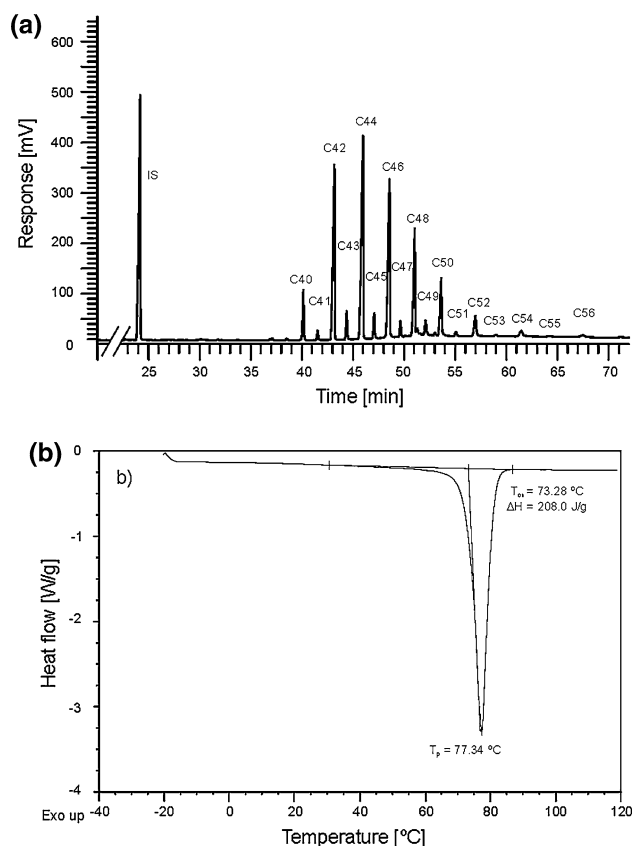
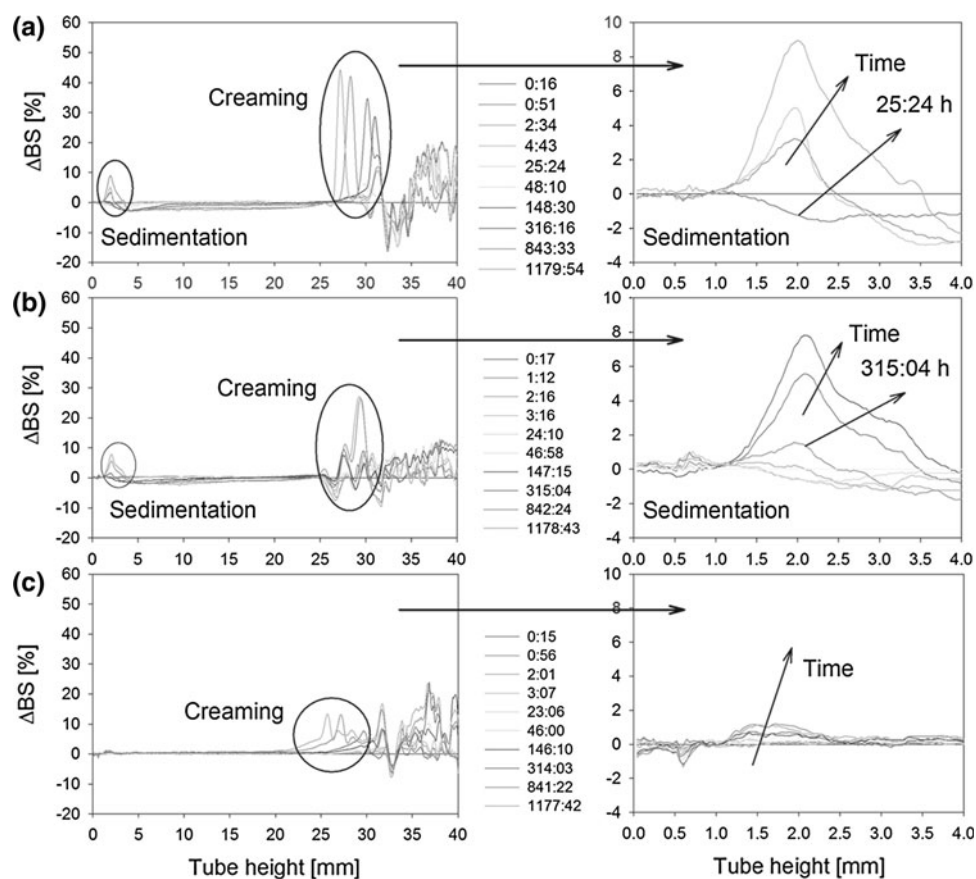


Fig. 1 Chromatogram (a) and DSC thermogram (b) of the sunflower waxes

temperature to determine the morphology of the wax crystals formed. Polarized micrographs were obtained using an Olympus BX-31 microscope (Olympus America, Melville, NY, USA) equipped with a digital camera (Infinity 2; Lumenera Scientific, Ottawa, ON, Canada) and $\times 10$ and $\times 20$ objective lens.

Fig. 2 BS profile of SFW 0.2 % emulsions with zoom over sedimentation peak: **a** PA 1 %, **b** PA 2 %, **c** PA 3 %. These data are represented as a function of time (different lines, hours:minutes) and of sample height (X-axis = 0–40 mm)



In order to measure crystals size, approximately 200 crystals were counted in three polarized micrographs of each emulsion. Crystals that appeared to be agglomerated or that were present in the borders of the micrographs were discarded to avoid incorrect measurements. The crystals were considered as spheres and the longest dimension as the diameter that was used to quantify the crystal sizes. The average crystal size was determined using image analysis software for PC, Pro Version 3.0 (1986–2000 Soft Imaging System).

Rheology

The rheological characteristics of the SFW/PA emulsions were determined using an AR-G2 controlled stress dynamic rheometer (TA Instruments) with concentric cylinder geometry. The cylinder used was an AR-G2 Peltier concentric cylinder recessed with Smart Swap Peltier temperature-controlled jacket (rotor radius = 14 mm, rotor height = 42 mm, cup radius = 15 mm, sample volume = 6.65 mL). The tests were carried out at 25 °C in ascending manner and without pre-shear. The range of shear rate $\dot{\gamma}$ was between 1×10^{-4} and 100 s^{-1} .

Film Preparation

Approximately 30 g of SFW/PA emulsion were poured into 9-cm Petri glass plates and allowed to dry at 50 °C

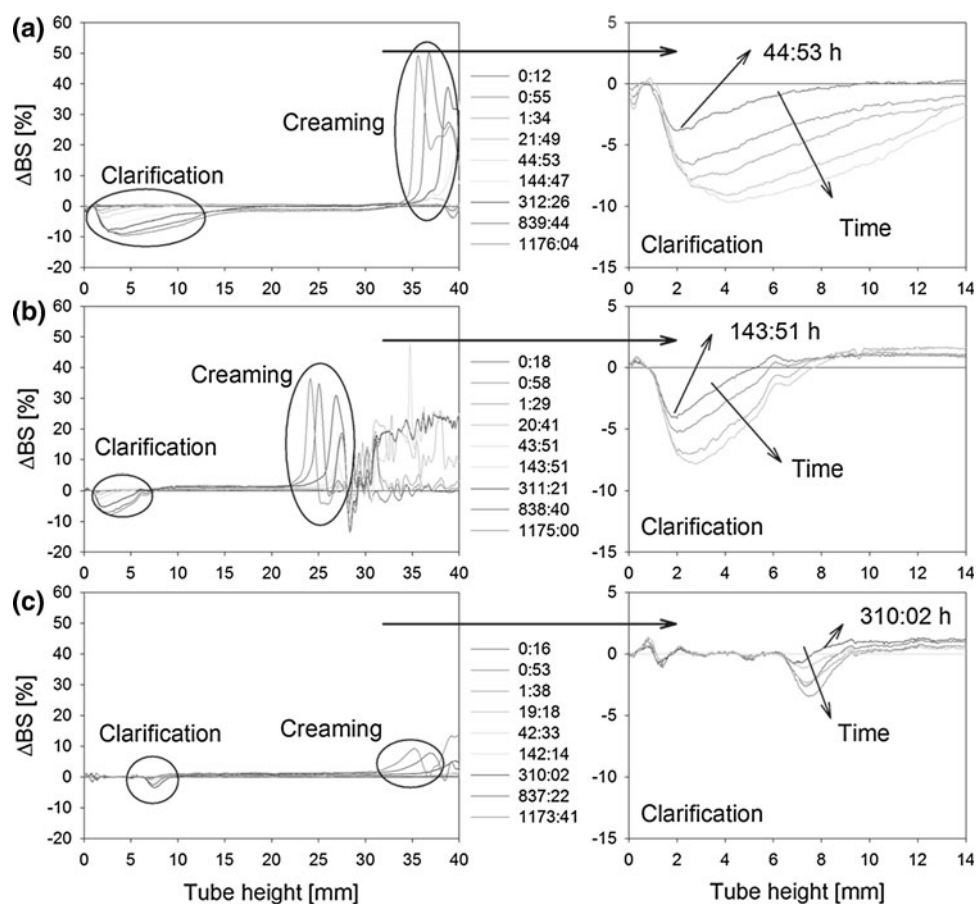
overnight. As the water evaporated from the SFW/PA emulsion, a thin film of pectin and SFW was deposited on the surface of the Petri dish. For film cross-linking, the Petri glasses with a thin film of pectin and SFW were immersed in approximately 30 g of a 2 % calcium chloride solution and allowed to stand for 30 min. The calcium reacts with the galacturonic acid in the pectin forming a cross-linked water-insoluble calcium pectinate film. The Petri dishes containing the cross-linked pectin film with SFW were rinsed three times with distilled water. After being peeled from the dishes, the SFW/PA films were stored at a known relative humidity.

Statistical Analysis

Emulsions with different concentrations of pectin and SFW were prepared in duplicate as described above. All the determinations in each independent sample were also performed in duplicate. Mean values and standard deviation were reported.

Significant differences were analyzed using ANOVA (two- and three-way tests) and a Holm-Sidak post hoc test ($\alpha = 0.05$). Statistical analysis was performed using Statgraphics statistical analysis software for PC, Sigma Plot for Windows v.8.01 (2002; SPSS, Chicago, IL, USA).

Fig. 3 BS profile of SFW 0.4 % emulsions with zoom over clarification peak: **a** PA 1 %, **b** PA 2 %, **c** PA 3 %. These data are represented as a function of time (different lines, hours:minutes) and of sample height (X-axis = 0–40 mm)



Results And Discussion

Wax Analysis

Table 1 and Fig. 1a show the chemical composition profile of SFW in terms of their carbon number. The wax fraction consisted of C40–C56 waxes with higher percentages of C46, C44 and C48, followed by C50 and C52, in accordance with previous studies [14, 20]. Bäumler et al. [14] found a similar wax profile analyzing the waxes present in the hulls of sunflower seeds obtained by sunflower seed hexane washing. This is in agreement with the fact that the hull contribution to the wax content in sunflower oil reaches approximately 80 % when only the crystallized fraction is considered [14, 21]. The quantitative analysis of SFW showed that the wax esters represented about 75 % of this material (754,229 mg/kg; Table 1; Fig. 1a). The remaining 25 % consisted of triacylglycerols and minor components from the crude oil. Sindhu Kanya et al. [22] reported 16.2 % of free fatty acids, 12.6 % of fatty alcohols, 66.0 % fatty esters, 6 % hydrocarbons, and traces of triacylglycerides when analyzing purified sunflower seed waxes from oil refineries.

In addition, SFW was characterized by DSC obtaining the following melting profile: $T_{on} = 73.5 \pm 0.4$ °C,

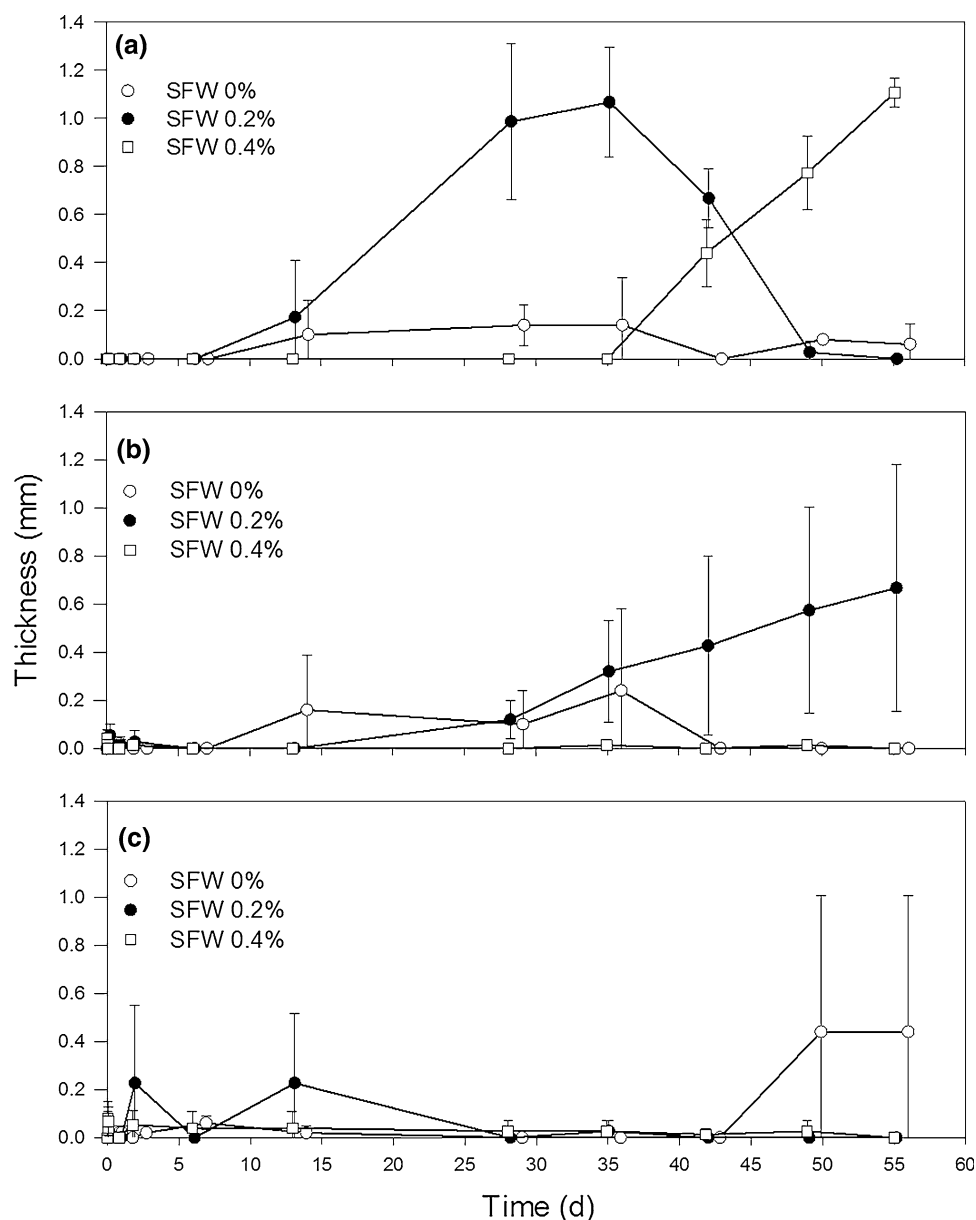
$T_p = 77.0 \pm 0.6$ °C and $\Delta H = 192.6 \pm 11.3$ J/g (Fig. 1b). The results are similar to those reported previously for purified sunflower waxes [20, 23]. Based on these data, SFW/PA emulsions were prepared at 80 °C to prevent wax crystallization during emulsification.

Emulsion Analysis

Stability

Colloids and dispersions are inherently unstable systems, but they can be considered as kinetically stable if their destabilization velocity is sufficiently low compared with the expected lifespan. Creaming occurs when the dispersed phase has a lower density than the continuous phase. The backscattering flux decreases at the bottom of the sample due to a decrease in the particle concentration in this part (clarification), whereas it increases at the top of the sample due to an increase in the concentration of the dispersed phase (creaming). Sedimentation is a similar phenomenon that takes place when the density of the dispersed phase is greater than that of the continuous phase. In this case, the backscattering increases at the bottom of the sample due to an increase in the concentration of the dispersed phase (sediment), whereas it

Fig. 4 Destabilization kinetics of emulsions: **a** PA 1 %, **b** PA 2 %, and **c** PA 3 %



decreases at the top of the sample due to a decrease in its concentration (clarification).

SFW were miscible in the film-forming solutions at 80 °C and easily mixed through a HIU generator, rendering milky and opaque emulsions with a nil transmission (T) signal. It should be pointed out that the backscattering (BS) signal can only be analyzed if the T signal is nil. Otherwise, the partial reflection of the light crossing the sample by the walls of the measurement cell would interfere with the BS signal. Pectin solutions without SFW presented a T signal (80–50 %) that decreased with increasing pectin content (data not shown).

Representative BS profiles obtained with the vertical scan macroscopic analyzer for each SFW/PA emulsion

containing 0.2 and 0.4 % SFW are presented in Figs. 2 and 3. The Y-axis represents the BS change with respect to the initial reading (Δ BS %), and the X-axis represents the sample height (mm) with respect to the cell bottom. After 25 h from the emulsion preparation a sedimentation process was observed in the emulsions containing 1 % pectin at 0.2 % SFW content (Fig. 2a). The line where an evident destabilization process at the bottom of the tube was observed is marked with an arrow which also indicates the specific time point associated to that onset of destabilization.

For emulsions with 0.4 % of SFW, the destabilization was characterized by clarification peaks at the bottom of the tube (Fig. 3a, tube height = 2–12 mm; Fig. 3b, c, tube height = 2–10 mm). Creaming peaks at the top of the tube

Fig. 5 Polarized light micrographs of: **a** SFW 0 %/PA 1 %, **b** SFW 0 %/PA 2 %, **c** SFW 0 %/PA 3 %, **d** SFW 0.2 %/PA 1 %, **e** SFW 0.2 %/PA 2 %, **f** SFW 0.2 %/PA 3 %, **g** SFW 0.4 %/PA 1 %, **h** SFW 0.4 %/PA 2 %, and **i** SFW 0.4 %/PA 3 %. Objective lens $\times 10$

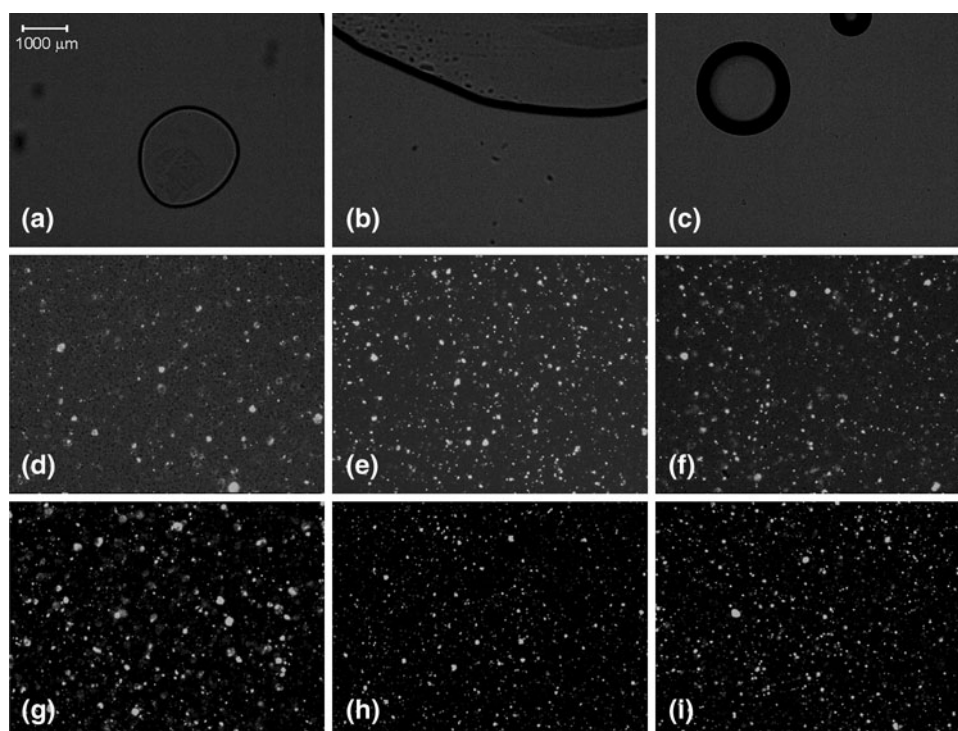


Table 2 Crystal size (μm) obtained for SFW/PA emulsions

Pectin % (wt/PA wt)	SFW % (wt/PA wt)	
	0.2	0.4
1	42.2 ± 1.8^a	43.1 ± 1.5^a
2	43.8 ± 1.2^a	32.3 ± 1.4^b
3	30.5 ± 1.5^b	31.0 ± 1.6^b

Mean value \pm confidence interval at $\alpha = 0.05$

Values in the same column followed by different letters are significantly different ($p < 0.05$) by the Holm-Sidak method

were observed in all the formulations. No clarification or sedimentation peaks were observed in emulsions with 0.2 % SFW and 3 % PA (Fig. 2c). Only small peaks were observed in emulsions with 0.4 % SFW and 3 % PA contents (Fig. 3c), indicating a very slight clarification and creaming process.

By comparing Fig. 2a–c and Fig. 3a–c, it is possible to observe that the displacements in the ΔBS % readings were less intense or even not present when pectin content increased. This is a clear indication that emulsion stability is affected by SFW and pectin content. For a constant SFW concentration, the higher the amount of pectin, the more stable the emulsion seemed to be.

Figure 4 shows the destabilization kinetics of the samples. Even though BS is not the best technique to measure semi-transparent samples, we decided to use it for 0 % SFW formulations to allow for comparison of the

treatments. It is evident from these figures that the emulsions with SFW were stable for almost 10 days from the initial reading. The higher destabilization kinetics were observed in emulsions made with 1 and 2 % PA (Fig. 4a, b). Taking into account SFW content, emulsions with higher SFW concentrations were more stable, regardless of the PA content. In Fig. 4b, c, it is clearly evident that the destabilization process is delayed or even completely inhibited in emulsions with higher pectin content. This fact could be attributed to the higher emulsion viscosity that stopped the sedimentation process; moreover, it is known that pectin can play a role as an emulsifier [6–9].

In the case of the 0.2 % SFW/1 % PA emulsion (Fig. 4a), it is possible to observe that the destabilization mechanism started after 10 days from the initial reading, reaching a maximum at 35 days, and after that the destabilization kinetics started to decrease. The fact that this phenomenon was not observed at higher pectin concentrations could be due to the solution viscosity and/or the emulsion stabilization effect of pectin that could hinder the crystallization of the sunflower wax. Firstly, the lower viscosity of 0.2 % SFW/1 % PA emulsion could affect the particle properties of polymorphism and polydispersion. Some authors showed that the esters of some alcohols with an odd number of carbon atoms presented different morphologies depending on the cooling conditions, the wax concentration in the oil [24–27] droplet size, and the nature of the emulsifier [28–30]. It is often assumed that an emulsified fat crystallizes via a homogeneous mechanism,

Fig. 6 Micrograph of SFW 0.4 %/PA 3 % emulsion: **a** non-polarized, **b** polarized. *Circles* indicate wax crystals inside droplets. Objective lens $\times 20$

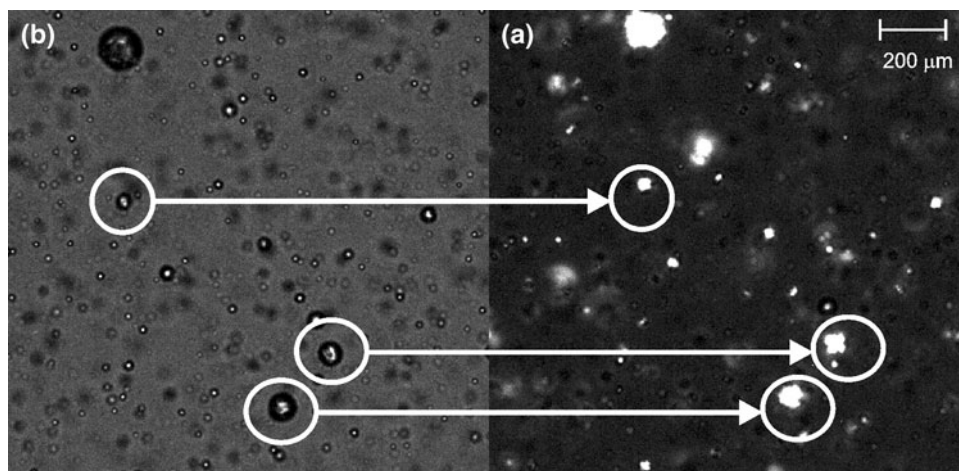


Fig. 7 Shear stress of SFW/PA emulsions vs shear rate, $T = 25\text{ }^{\circ}\text{C}$. Pectin and SFW content analysis: **a** PA 1 %, **b** PA 2 %, **c** PA 3 %, **d** SFW 0.0 %, **e** SFW 0.2 %, and **f** SFW 0.4 %

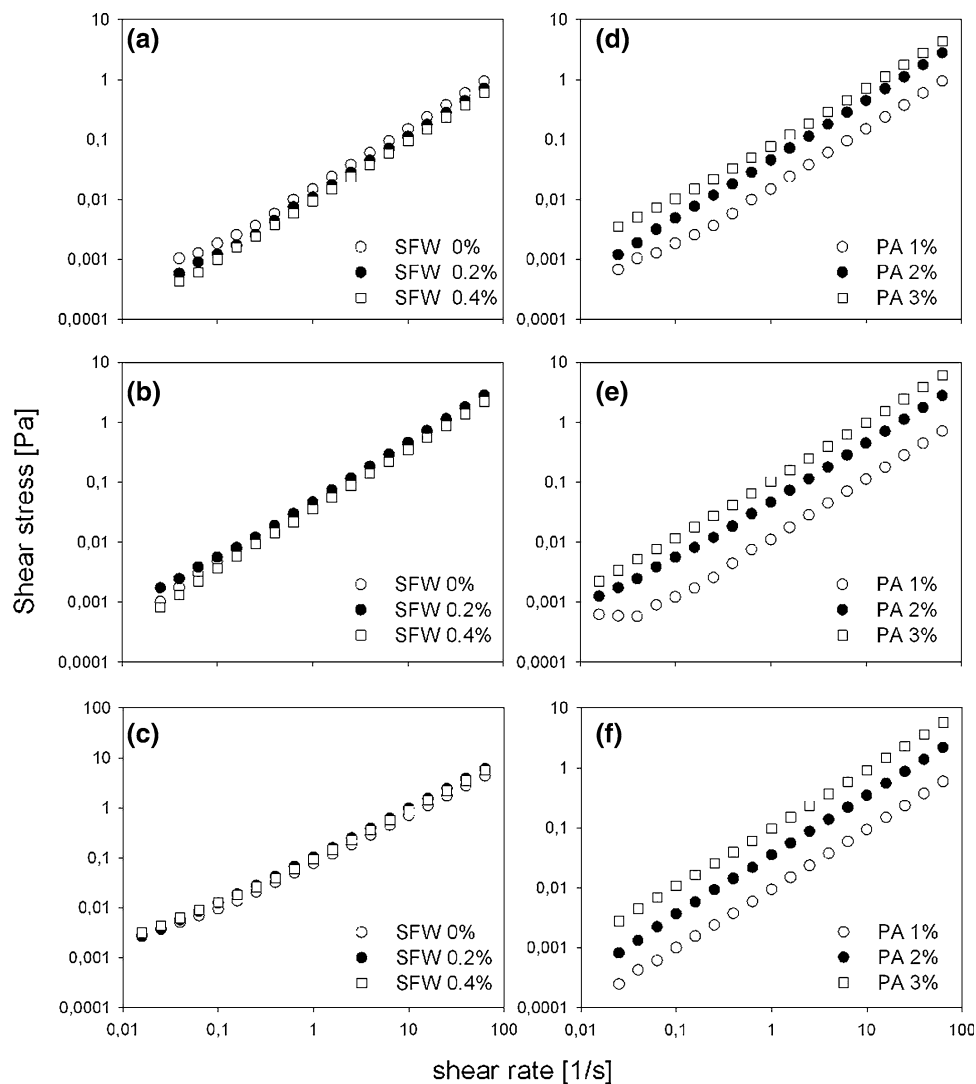


Table 3 Rheological parameters of the SFW/PA emulsions, $T = 25\text{ }^{\circ}\text{C}$

Composition	SFW %/PA % (wt/wt)								
	1/0	1/0.2	1/0.4	2/0	2/0.2	2/0.4	3/0	3/0.2	3/0.4
Newton model									
η (Pa s)	0.01482	0.01116	9.39×10^{-3}	0.04367	0.04431	0.03456	0.06828	0.09584	0.09032
Error	0.3915	0.4446	0.7175	1.22	0.7662	0.175	2.099	1.566	1.581
Oswald-de Waele model									
K (Pa s)	0.01605	0.01104	9.21×10^{-3}	0.04517	0.04524	0.0347	0.07288	0.1001	0.09156
n	0.9982	1.002	1.004	0.9922	0.9922	0.9991	0.9868	0.9901	0.9889
Error	0.2456	0.2635	0.2547	0.1351	0.17	0.1009	0.4128	0.2942	0.3786
Herschel-Buckley model									
τ_y (Pa)	2.69×10^{-4}	3.50×10^{-4}	1.39×10^{-5}	4.55×10^{-4}	5.83×10^{-4}	4.25×10^{-5}	2.01×10^{-3}	6.96×10^{-4}	1.67×10^{-3}
K (Pa s)	0.01601	0.01099	9.20×10^{-3}	0.04509	0.04516	0.03469	0.07429	0.1	0.09128
n	0.9986	1.003	1.004	0.9926	0.9958	0.9992	0.9849	0.9903	0.9896
Error	0.2081	0.2422	0.2491	0.1483	0.1396	0.1004	0.4904	0.2935	0.3724
Casson model									
τ_y (Pa)	2.70×10^{-7}	6.66×10^{-7}	9.61×10^{-8}	1.32×10^{-4}	6.74×10^{-5}	1.75×10^{-6}	8.93×10^{-4}	5.03×10^{-4}	4.74×10^{-4}
K (Pa s)	3.23×10^{-4}	1.59×10^{-4}	1.15×10^{-4}	0.04308	0.04392	0.03532	0.06652	0.09424	0.08884
Error	366.6	368.6	369.2	0.5476	0.4336	0.156	1.131	0.7927	0.7946

Values represent the model parameters with the minor error obtained of three replicates

Table 4 Apparent viscosity of the SFW/PA emulsions, $T = 25\text{ }^{\circ}\text{C}$

Pectin % (wt/wt)	SFW % (wt/wt)		
	0	0.2	0.4
1	0.0154 ± 0.0008^a	0.0110 ± 0.001^{ab}	0.0095 ± 0.0066^b
2	0.0456 ± 0.0007^c	0.0465 ± 0.0112^c	0.0361 ± 0.0088^d
3	0.0758 ± 0.0081^e	0.1031 ± 0.0110^f	0.0951 ± 0.0074^g

Mean value \pm standard deviation ($n = 2$)

Values followed by different letters are significantly different ($p < 0.05$) by the Holm-Sidak method

but in fact homogeneous nucleation will only dominate if the particle size is reduced, so there is much less than one nucleus per droplet and hence the dominant mechanism may depend on the composition and purity of the fat as well as the structure of the emulsion. Recently, the effects of polydispersity on the crystallization behavior of emulsified fats have been considered, showing that the crystallization rate should increase with polydispersity [31]. Secondly, it is possible that pectin acts as an emulsifier following the model of association proposed by Leroux et al. [9]. According to this model, the pectin molecules surround the molten wax droplet causing the inhibition of both the nucleation and growth of crystals. As a result, a decrease in the wax crystal size is observed.

The results shown in this paper suggest that wax concentration has some effect on the destabilization kinetics, but further research is necessary to evaluate whether the changes in BS as a function of time are due to different crystal or clusters morphologies.

Microscopy

Polarized light micrographs (PLM) obtained for SFW/PA emulsions are shown in Fig. 5. Wax crystals were observed in all the cases, with size ranging between 31 and 44 μm . Smaller and fewer crystals were observed with increasing amounts of pectin (Table 2). This observation is agreement with our previous discussion about the emulsifying capacity of pectins. A higher amount of pectin in the emulsions resulted in smaller droplets that entrapped the wax material, which crystallized inside the droplets upon cooling. The differences in crystal size shown in this section could indicate the presence of different crystals or clusters morphologies. However, further morphological studies are required in order to confirm it.

The same crystals arrangement was observed in all the emulsions. Crystals were located radially around a center point. Figure 6a, a non-polarized micrograph obtained for the emulsion formulated with 3 % pectin and 0.4 % SFW,

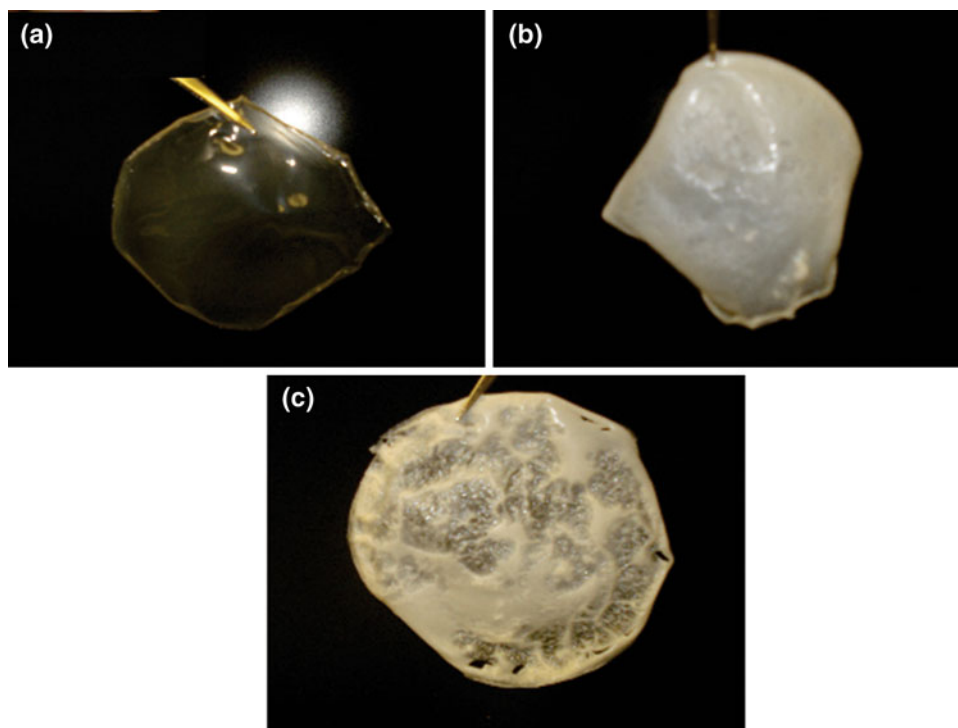


Fig. 8 Photograph showing the appearance of films prepared from calcium pectinate (a), 0.2 % SFW/PA (b) and 0.4 % SFW/PA (c), respectively

shows wax crystals entrapped inside droplets. Similar shapes were observed for wax of crude oils (concentrations approx. 0.15 %), where the crystals were clustered in shapes assimilated to a sphere [25]. Figure 6b presents a polarized micrograph of the same sample where it is possible to appreciate the SFW crystal pattern.

Rheology

The shear stress versus shear rates relationship for the studied SFW/PA emulsions are shown in Fig. 7. A regression analysis was conducted to verify the applicability of the rheological models. The following rheological models were evaluated: Newton $\tau = K\dot{\gamma}$, Oswald-de Waele $\tau = K\dot{\gamma}^n$, Herschel–Bulkley (H–B) $\tau = \tau_y + K\dot{\gamma}^n$, and Casson $\tau = \tau_y^{1/2} + K\dot{\gamma}^{1/2}$, where τ is the shear stress, $\dot{\gamma}$ is the shear rate, η is the fluid viscosity in the Newton model, K is the consistency index, n is the flow behavior index, and τ_y is the yield stress.

In Fig. 7, it is possible to observe that the shear rate/shear stress data results in a straight line with an apparent yield stress. The H–B model takes into account the occurrence of yield stress, and it is therefore more appropriate to explain the rheological behavior of the emulsions in all the shear stress range considered (see Table 3). This assumption is consistent with the oscillatory test results reported by Lopes da Silva et al. [32], who suggested the

existence of a yield stress. Other authors also considered the H–B rheological model as the most adequate for pectin solutions [13, 33].

The magnitude of the yield stress will determine the thickness of the coating on a vertical surface. No interactions between different pectin and SFW content values were found ($p = 0.140$, $\alpha = 0.05$). In almost all the SFW/PA emulsions, the highest values for τ_y corresponded to the solution with the highest amount of pectin ($p < 0.05$), and the addition of SFW did not cause significant differences ($p = 0.068$). Apparent viscosities obtained using the H–B model are shown in Table 4. This data was analyzed with a 2-way ANOVA, with pectin and SFW content as factors. Significant differences ($\alpha = 0.05$) were found between treatments and their interactions ($p \ll 0.001$). The increase in pectin content resulted in higher viscosity values ($p \ll 0.001$) (Table 4). However, the effect of the SFW addition was not so evident, showing a small decrease in viscosity for 1 and 2 % pectin content ($p < 0.05$), but the opposite effect for 3 % pectin ($p < 0.05$) (Table 4).

Film Preparation

In order to confirm the capacity of SFW/PA emulsions to produce edible films, some films were elaborated in our laboratory. The films were easily prepared over a period of approximately 24 h. The films obtained were flexible and

adequate for handling and wrapping. SFW-free films were transparent (Fig. 8a), in contrast with SFW/PA films (Fig. 8b, c), which were opaque. Films made from 0.2 % SFW/2 % PA emulsion showed an adequate inclusion of the lipid material (Fig. 8b), while films with a 0.4 % SFW content had irregular surfaces with amorphous areas showing non-homogeneity (Fig. 8c). The analysis of the optical and rheological properties, water resistance, and thermal behavior of SFW/PA is the objective of a forthcoming work.

Conclusion

The present study demonstrated that film-forming solutions containing sunflower-oil waxes and low-methoxyl citric pectin present adequate physical properties to be used in the elaboration of edible films. The characterization of these solutions is useful to optimize the production of pectin-wax-based emulsion coatings to prolong the shelf-life of food products. Results shown in this study contribute to the search of food applications for by-products of vegetable oil refining industries, in order to increase their commercial value.

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References

- Thakur BR, Singh RK, Handa AK, Rao MA (1997) Chemistry and uses of pectin—a review. *Crit Rev Food Sci Nutr* 37:47–73
- Hamilton RJ (1995) Commercial waxes: their composition and applications in waxes: chemistry molecular biology and functions. *Oily*, Dundee, pp 131–156
- Chick J, Hernandez RJ (2002) Physical, thermal, and barrier characterization of casein-wax-based edible films. *Food Engin Phys Prop* 67(3):1073–1079
- Kim K, Hwang K, Weller C, Hanna M (2002) Preparation and characterization of soy protein isolate films modified with sorghum wax. *J Am Oil Chem Soc* 79:615–619
- Dicharry C, Arla D, Sinquin A, Graciaa A, Bouriat P (2006) Stability of water/crude oil emulsions based on interfacial dilatational rheology. *J Colloid Interface Sci* 297:785–791
- Rooker W (1927) New uses of fruit pectin. *Fruit Products J Am Vineg Ind* 7(1):11
- Kertesz Z (1951) *The pectic substances*. Interscience, New York
- Parker A, Boulenguer P, Kravtchenko T (1994) Effect of the addition of high methoxyl pectin on the rheology and colloidal stability of acid milk drinks. In: Nishinari K, Doi E (eds) *Food hydrocolloids: structure, properties and functions*. Plenum, New York, p 307–312
- Leroux J, Langendorff V, Schick G, Vaishnav V, Mazoyer J (2003) Emulsion stabilizing properties of pectin. *Food Hydrocoll* 17:455–462
- Pastor Navarro C (2010) *Recubrimientos Comestibles a Base Dehidroxipropil Metilcelulosa: Caracterización y Aplicación*. Thesis, Polytechnic University of Valencia
- Lewis MJ (1993) *Propiedades físicas de los alimentos y de los sistemas de procesamiento*. Editorial Acribia S.A, Zaragoza
- Gündüz U (2003) Evaluation of viscosities of aqueous two-phase systems containing protein. *J Chromatogr* 807:157–161
- Panchev I, Nikolova KR, Pashova S (2009) Physical characteristics of wax containing pectin aqueous solutions. *J Optoelectron Adv Mater* 11:1214–1217
- Bäumler ER, Crapiste GH, Carelli AA (2007) Sunflower-oil wax reduction by seed solvent washing. *J Am Oil Chem Soc* 84:603–608
- Martini S, Tippetts M (2008) Effect of processing conditions on the crystallization behavior and destabilization kinetics of oil-in-water emulsions. *J Am Oil Chem Soc* 85(2):119–128
- Herrera ML (2012) *Analytical techniques for studying the physical properties of lipid emulsions. Briefs in food, health, and nutrition, vol 3*. Springer, New York
- Mengual O, Meunier G, Cayré I, Puech K, Snabre P (1999) TURBISCAN MA 2000: Multiple light scattering measurement for concentrated emulsion and suspension instability analysis. *Talanta* 50:445–456
- Tippetts M, Martini S (2009) Effect of oil content and processing conditions on the thermal behavior and physicochemical stability of oil-in-water emulsions. *Int J Food Sci Technol* 44:206–215
- Tippetts M, Martini S (2009) Effect of cooling rate on the thermal behaviour and physicochemical stability of oil-in-water emulsions. *Food Res Int* 42:847–855
- Martini S, Carelli AA, Lee J (2008) Effect of the addition of waxes on the crystallization behavior of anhydrous milk fat. *J Am Oil Chem Soc* 85:1097–1104
- Carelli AA, Frizzera LM, Forbito PR, Crapiste GH (2002) Wax composition of sunflower seed oils. *J Am Oil Chem Soc* 79:763–768
- Sindhu Kanya TC, Jaganmohan Rao L, Shanthakka Sastry MC (2007) Characterization of wax esters, free fatty alcohols and free fatty acids of crude wax from sunflower seed oil refineries. *Food Chem* 101:1552–1557
- Martini S, Añón MC (2003) Crystallization of sunflower oil waxes. *J Am Oil Chem Soc* 77:1087–1092
- Aleby S, Fischmeister I, Iyengar B (1971) The infrared spectra and polymorphism of long chain esters: iv. some esters from tetradecanol, hexadecanol, octadecanol, eicosanol, docosanol and dodecanoic, tetradecanoic, hexadecanoic octadecanoic and eicosanoic acid. *Lipids* 6:421–425
- Rivarola G, Añón M, Calvelo A (1985) Crystallization of waxes during sunflowerseed oil refining. *J Am Oil Chem Soc* 62:1508–1513
- Özilgen S, Simoneau C, German JB, McCarthy MJ, Reid DS (1993) Crystallization kinetics of emulsified triglycerides. *J Sci Food Agric* 61(1):101–108
- Simoneau C, McCarthy MJ, Reid DS, German JB (1993) Influence of triglyceride composition on crystallization kinetics of model emulsions. *J Food Eng* 19(4):365–387
- Skoda W, Van den Tempel M (1963) Crystallization of emulsified triglycerides. *J Coll Sci* 18(6):568–584
- Krog N, Larsson K (1992) Crystallization at interfaces in food emulsions: a general phenomenon. *Fat Sci Technol* 94(2):55–57
- Vanapalli SA, Palanuwech J, Coupland JN (2002) Stability of emulsions to dispersed phase crystallization: effect of oil type, dispersed phase volume fraction, and cooling rate. *Colloids Surf* 204:227–237
- Coupland JN (2002) Crystallization in emulsions. *Curr Opin Colloid Interface Sci* 7:445–450

32. Lopes da Silva J, Gonçalves M, Rao M (1993) Viscoelastic behaviour of mixtures of locust bean gum and pectin dispersions. *J Food Eng* 18(3):211–228
33. Dolz M, Hernández M, Cabeza C, Casanovas A, Delegido J (2004) Analysis of stability of food emulsions by Eyring's theory: influence of different biopolymers. *J Appl Polym Sci* 92:2653–2657