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Stability of oil-in-water (O/W) emulsions with chia (*Salvia hispanica* L.) mucilage

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ABSTRACT

The stability of oil-in-water (O/W) emulsions prepared with different concentrations of chia mucilage extracted by two different methods was evaluated as a function of refrigerated storage time. O/W emulsions (20:80 wt/wt) formulated with refined corn oil, chia mucilage dispersions (0.25, 0.50, 0.75 and 1.00% wt/wt) and 0.1% wt/wt of Tween 80 were stored at 4 ± 1 °C for 120 days, evaluating their stability periodically. An emulsion without added mucilage was also prepared as a control. The emulsions were characterized determining the flow behavior, the evolution of the backscattering profiles (%BS), particle size distribution, and microscopic observations. Viscosity increased as increasing mucilage concentration, revealing a pseudoplastic flow behavior. The most stable emulsions during storage were those with ≥0.75% chia mucilage concentration, and those with mucilage obtained by Method II (which presents a higher level of purity than MI mucilage). All the emulsions prepared with mucilage obtained by Method II presented smaller droplet diameters ([D4,3]) than those prepared with mucilage obtained by Method I. Particle size distribution did not show important variations as a function of time, concentration and type of mucilage, presenting in general a unimodal behavior. The results suggested that the addition of chia mucilage (≥0.75%) to O/W emulsions improved their stability against gravitational phase separation by increasing the viscosity of the aqueous phase, limiting the mobility of the oil droplets in the emulsions.

1. Introduction

Chia seeds (*Salvia hispanica* L.) contain 5–6% mucilage that can be used as soluble dietary fiber, increasing the viscosity of the chyme, slowing the stomach emptying and increasing the feeling of satiety (Hentry, Mittleman, & McCrohan, 1990; Reyes-Caudillo, Tecante, & Valdivia-López, 2008). Chia mucilage begins to be exuded from seeds as soon as they are placed in water. Different extraction methods have been studied, including various seed pretreatments, agitation techniques, seed:water ratios, soaking times and temperatures (Coorey, Tjoe, & Jayasena, 2014; Muñoz, Cobos, Diaz, & Aguilera, 2012a; Segura-Campos, Acosta-Chi, Rosado-Rubio, Chel-Guerrero, & Betancur-Ancona, 2014). The structural units of this anionic heteropolysaccharide consist of xylose (Xyl) and glucose (Glc) as the main sugars with xylose-to-glucose ratio of approximately 2:1. A significant amount of uronic acids (glucuronic acid and galacturonic acid) and two other neutral sugars, namely arabinose (Ara) and galactose (Gal), were also detected (Timilsena, Adhikari, Kasapis, & Adhikari, 2016). Mucilage often contains a small amount of proteinaceous material as an integral part of its structure that can be adsorbed on the oil-water interface (Ávila-de la Rosa, Álvarez-Ramírez, Vernon-Carter, Carrillo-Navas, & Pérez-Alonso, 2015). The available data on the functional properties of chia mucilage are recent, and they indicate that it is a polymer with thickening properties (Lin, Daniel, & Whistler, 1994). In 1996, FAO described it as a potential source of polysaccharide gum because of its exceptional mucilaginous properties at low concentration in aqueous solutions (Hulse, 1996). Given its high solubility in water (66%w/v at 25 °C), chia mucilage could become an important ingredient in the food industry, since it is considered that gums with higher solubility have a better quality (Capitani, Ixtaina, Nolasco, & Tomás, 2013; Mhinzi & Mrosso, 1995). Several studies have reported on the rheological properties of

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different aqueous dispersions of chia mucilage, and on its potential to be used in the production of films and edible coatings (Capitani et al., 2015; Muñoz, Aguilera, Rodríguez-Turienzo, Cobos, & Diaz, 2012b; Timilsena, Adhikari, Kasapis, & Adhikari, 2015). Recent studies on the viscoelastic properties of chia mucilage dispersions at the vicinity of the interface of O/W emulsions reported that these properties depend on the distance from the interface as a consequence of the complex interactions between biomaterial particles. In addition, the results suggested the possible stratification of the mucilage material surrounding the oily phase, reflecting the emulsification and stabilization capabilities of chia mucilage (Ávila-de la Rosa et al., 2015). Timilsena et al. (2016) evaluated the emulsifying properties of purified chia mucilage (2.6% protein) in terms of the emulsion activity index and the emulsion stability index. They found that these parameters increased with increasing gum-oil ratio. They also observed a substantial decrease in the surface tension of the mucilage in the air-water interface when the concentration increased up to 1 g/kg of suspension, with the decrease being slower at higher concentrations. Although the presence of a low concentration of protein contributes to the surface activity of the chia mucilage dispersions, as it was observed for other gums (Garti, Madar, Aserin, & Sternheim, 1997), the slower decrease in surface tension with increasing concentrations of the polysaccharide suggests steric phenomena (Timilsena et al., 2016). It is well known that the main mechanism of polysaccharides in the stabilization of O/W emulsions is based on their mainly hydrophilic structure, which gives them the capacity to increase the viscosity of the aqueous phase, thus reducing the mobility of the oil droplets hindering their collision (Garti & Leser, 2001; Vegi et al., 2009).

Although there is a number of studies on the effect of many polysaccharides, such as basil seed gum (Osano, Hosseini-Parvar, Matia-Merino, & Golding, 2014), *Lipidium perfoliatum* seed gum (Soleimanpour, Koocheki, & Kadkhodaee, 2013), *Alyssum homolocarpum* seed gum (Koocheki, Kadkhodaee, Mortazavi, Shahidi, & Taherian, 2009a), xanthan gum (Sun, Gunasekaran, & Richrads, 2007), fenugreek, locust bean, flax, oats, guar gum (Huang, Kakuda, & Cui, 2001) on the stability of O/W emulsions, few reports were found in the literature on the use of chia mucilage in the formulation of this food type (Ávila-de la Rosa et al., 2015; Guiotto, Capitani, Nolasco, & Tomás, 2016). Therefore, the aim of this work was to study the effect of adding different concentrations of chia mucilage obtained by two different methods on the stability of O/W emulsions, as a function of refrigerated storage time.

2. Materials and methods

2.1. Materials

The commercial chia seeds used in this study were obtained from Salta, Argentina (25 °S 65.5 °W). The seeds were cleaned manually and the foreign matter, such as granules, dirt and broken seeds, was removed. Then they were packaged in hermetic plastic containers and stored at 5 ± 1 °C until further use.

2.2. Obtaining the chia mucilage

The mucilage was obtained from whole chia seeds by two methods:

2.2.1. Method I (MI)

Samples of 10 g of whole seeds were placed in a tray (9 cm × 14 cm × 5 cm), and distilled water was added at a 1:10 (wt/vt) ratio. They were covered with aluminum foil and maintained at room temperature for 4 h. Then, the samples were frozen at −20 °C for 96 h, followed by freeze-drying (−50 °C, 0.033 mbar, 4d) (RIFICOR freeze dryer, Argentina). The dried mucilage was separated from the seeds by rubbing in a ZONYTEST stirrer (Buenos Aires, Argentina) over a 20 ASTM mesh screen (840 μm) for three periods of 15 min each (Capitani et al., 2013).

2.2.2. Method II (MII)

Mucilage was obtained by the procedure proposed by Marin Flores, Acevedo, Tamez, Nevero, & Garay (2008) with modifications proposed by Capitani et al. (2015): whole chia seeds were soaked in water (1:20 wt/vt) for 1 h at room temperature with manual stirring in order to induce the mucilage exudation. The extracted mucilage was separated from the seeds by vacuum filtration through a mesh (100 μm) at 220 mbar. Then the mucilage solution was concentrated on a rotavapor (Büchi R-215, Switzerland) at 55 °C under vacuum. It was frozen at −20 °C for 96 h followed by freeze-drying (−45 °C, 0.060 mbar, 5 d) (LABCONCO freeze dryer, Freezone 18, USA). The dried mucilage was ground using a food processor (Moulinex, model 1736249, Spain) to obtain a fine powder.

Both types of mucilage were packaged in hermetically sealed plastic containers, and stored in a desiccator to preserve them from humidity.

2.3. Proximate composition of mucilages

AOCS (1998) procedures were used to analyzemoisture (Ba 2a-38 method), crude fiber (Ba 6–84 method) and ash (Ba 5a-49 method) contents. Oil content was determined following the IUPAC Standard Method 1.122 (1992). Total nitrogen content (N) was determined by Kjeldahl method according to AOAC (1990), and the protein content was calculated as nitrogen x 6.25. Carbohydrate content was estimated as nitrogen-free extract (NFE) by difference using Equation (1):

$$NFE = 100 - (oil + protein + crude\ fiber + ash) \quad (1)$$

2.4. Preparation of aqueous dispersions

The dispersions were prepared by adding 0.25, 0.50, 0.75 and 1.00 g dried mucilage into 80 g deionized water while stirring at 60 °C for 30 min. The dispersions were then cooled to room temperature and left overnight at 4 °C (to ensure complete hydration) prior to use in emulsion preparation.

2.5. Oil-in-water (O/W) emulsion preparation

Oil-in-water (O/W) emulsions (20:80 wt/wt) were prepared with refined corn oil (Arcor, Argentina), different dispersions of chia mucilage, 0.1% wt/wt of Tween 80 and 0.01%wt/wt of sodium azide to prevent bacterial growth. For both of the emulsions formulated with MI and MII mucilage, the continuous phase was prepared to obtain a final composition of emulsions with mucilage concentrations of 0.25, 0.50, 0.75 and 1.00% wt/wt. An emulsion without added mucilage was also prepared as a control. The emulsions were prepared at room temperature in an Ultraturrax T-25 homogenizer (Janke & Kunkel, IKA-Labortechnik, Germany) using an S 25 N-10 G dispersing tool (rotor diameter 7.5 mm) at 9500 rpm, for 1 min. The resulting pre-emulsions were then treated for 3.5 min with an ultrasonic processor (model VCX 750, Sonics & Materials, Inc., USA) at full power (750 W), with a titanium sonotrode (13 mm in diameter) immersed 1 cm below the surface of the liquid. The temperature was kept constant at 25 ± 1 °C throughout sonication by a bath of water and ice. A thermometer was placed

inside the sanitation chamber to control the temperature rise during sonication. The initial pH of the emulsions was measured in duplicate.

2.6. Characterization of emulsions

2.6.1. Rheological properties

The flow behavior was determined by the procedure proposed by Guiotto et al. (2016) with a Haake RS600 controlled stress rheometer (ThermoElectron, Germany) using PP35-S serrated parallel plate measuring geometry (35 mm diameter), and measurements were performed at 25 ± 1 °C in the 1–500 s^{-1} range. The experimental data (shear stress-shear rate) were fitted using the Power Law model according to Equation (2):

$$\tau = k\dot{\gamma}^n \quad (2)$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), k is the consistency index (Pa s^n) and n is the flow behavior index (dimensionless).

2.6.2. Emulsion stability

Global stability of the emulsions was determined as a function of storage time and the height of the sample in the tube (about 65 mm) by measurements of dispersed light with a QuickScan Vertical Optical Analyzer (Beckman Coulter, Fullerton, USA), as described by Pan, Tomás, & Añon (2002). The destabilization kinetics was studied by recording the mean values of backscattering (BS) in two areas of the tube: bottom zone (Zone I, 10–20 mm), and upper zone (Zone II, 45–55 mm).

2.6.3. Droplet size distribution

The droplet size distribution was determined by laser diffraction with a particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., UK) with a Hydro 2000MU dispersion unit. Approximately 1 mL of emulsion was suspended directly in a water bath (approximately 600 mL) of the dispersion system with a pump speed of 2000 rpm, reaching an obscuration of 15–18%, where a laser beam passes through a transparent internal cell where the diluted emulsion is recirculated. The light scattered at various angles from droplets of different sizes passes through a complex optical system, obtaining an angular light scattering pattern. The software of the equipment then translates this pattern into the corresponding particle size distribution (McClements, 1999). The refractive indices of sunflower oil (1.47) as particle, and water (1.33) as dispersant, were used. The DeBrouker mean diameter (D[4,3]) associated with volume droplet distribution was determined.

2.6.4. Microscopic observation

After preparation, the microstructure of each emulsion was observed at different times with a Leica DMLB optical microscope. Micrographs were taken with a Leica DC 100 camera (Leica Microscopy Systems Ltd., Heerbrugg, Switzerland) with 63× magnification and at room temperature (25 ± 1 °C).

2.7. Storage of emulsions

Once prepared the emulsions were stored in a cold chamber at 4 ± 1 °C for 120 days. The physicochemical stability was evaluated immediately after preparation of emulsions and each week during storage time determining the flow behavior, the backscattering profiles, the D[4,3] diameter, and the microstructure as previously described.

2.8. Experimental design and statistical analysis

A 5 × 2 fully factorial design, replicated twice, was used to study the effects of mucilage concentration (0.00, 0.25, 0.50, 0.75 and 1.00% wt/wt) and the method of mucilage extraction (MI and MII) on each studied variable (backscattering profiles and mean diameter) independently for each time period of analysis. It should be noted that the parameters obtained for the Power Law model were statistically analyzed considering the emulsions with added chia mucilage, taking into account the observed flow behavior. Results were analyzed by a multifactorial ANOVA test (95% level of confidence) to study the main effects and the interactions between the factors. Means were separated according to Tukey's multiple comparison tests ($p < 0.05$) (95% level of confidence) in all cases. Statistical analysis was performed using the Infostat software (Infostat, 2004).

The influence of storage time on the physicochemical stability of emulsions was analyzed by an unifactorial ANOVA test (95% level of confidence).

3. Results and discussion

3.1. Proximate composition of mucilages

The two studied methods of mucilage extraction exhibited a similar yield of 3.8 ± 0.1 and 3.7 ± 0.1% (d.b.) for MI and MII, respectively. Protein and lipid content were significantly higher ($p < 0.05$) for MI, whereas crude fiber content was significantly higher for MII (Fig. 1). The differences observed between MI and MII can be attributed to the different methods used to separate the seeds from the liquid mucilage (in Method I the mucilage exudate was removed from the seeds by dry fractionation, whereas in Method II the mucilage solution was removed from the seeds by filtration), as well as to the differences in the values of the variables of the soaking stage (Capitani et al., 2015). Previous studies have shown that the protein content of mucilage extracted from *Lepidium perfoliatum* seeds (Koocheki, Taherian, Razavi, & Bostan, 2009b) and *Lepidium sativum* seeds (Karazhiyan, Razavi, & Phillips, 2011) significantly increases by increasing the extraction time in the solubilization stage. In addition, the protein content of chia mucilage obtained by Method I was higher (18.8%) than that reported by Ávila-de la Rosa et al. (2015) and Guiotto et al. (2016) for chia mucilage (4.2 and 7.3%, respectively), who hydrated the

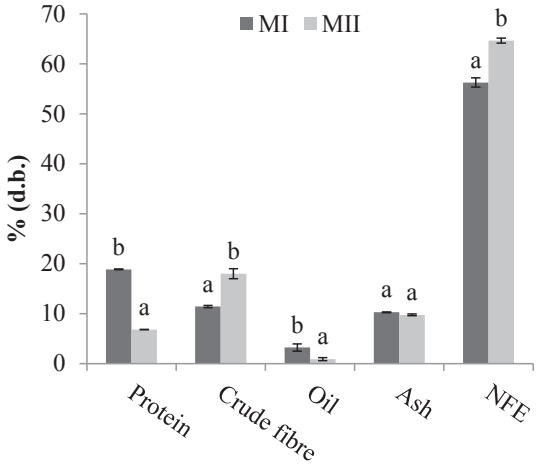

Fig. 1. Proximate composition (% d.b.) of chia mucilage obtained by different methods (MI and MII). Different letters indicate significant differences between samples for each component ($p < 0.05$). Each value is an average of three determinations ($n = 3$).

seeds for 2 and 1 h, respectively, and separated the seeds before the drying stage of the mucilage solution.

3.2. Rheological properties

The O/W emulsion without chia mucilage at initial time exhibited a typical Newtonian flow behavior ($n = 1.05 \pm 0.02$), characterized by a linear relationship between the applied shear stress and the shear rate (i.e., constant viscosity). The average viscosity (η) was of 0.0021 ± 0.00004 Pa s.

The variation of the apparent viscosity as a function of shear rate is shown in Fig. 2a and b for the different O/W emulsions at initial time, formulated with various concentrations of chia mucilage obtained by MI and MII, respectively. It is worth noting that at the other storage times no differences were observed with respect to initial time in the behavior of the apparent viscosity of the emulsions in relation with shear rate. The viscosity of the emulsions decreased as the shear rate increased, and this effect was more pronounced at low shear rates and when mucilage content increased in the aqueous phase of the emulsions. This fact can be explained due to the higher shear rates and a larger number of hydrocolloid molecules, the alignment of the droplets with the flow direction increases, offering a lower resistance, and thus a lower viscosity (McClements, 1999; Soleimanpour et al., 2013). On the other hand, the emulsions prepared with MII mucilage (Fig. 2b) exhibited a higher viscosity than those with MI (Fig. 2a) for all the studied concentrations. It should be noted that the viscosity of the emulsions increased with increasing mucilage concentration. Therefore, there were a larger proportion of high-molecular-weight molecules in the aqueous phase that would generate a greater flow resistance by inhibiting mobility of the droplets and frequency of collision, and that would favor the formation of a three-dimensional structure to prevent creaming (Nor Hayati, Che Man, Tan, & Nor Aini, 2009). A similar behavior was observed in O/W emulsions with different concentrations of gum extracted from *Alyssum homolocarpum* and *Lepidium perfoliatum* seeds (Koocheki et al., 2009a; Soleimanpour et al., 2013).

The values of the consistency index (k) and flow behavior (n) of all the studied emulsions with MI and MII corresponding to initial time are shown in Table 1. For the different emulsions, these parameters (k and n) varied significantly ($p < 0.05$) according to the type and concentration of mucilage used, without any significant interaction ($p > 0.05$) between the two factors studied. In all cases, n values were below 1, indicating a pseudoplastic flow behavior. The determination coefficients (R^2) were close to 1, showing that the power law model was adequate to determine the flow behavior of emulsions with chia mucilage obtained by different methods and at different concentrations. The emulsions formulated with MII mucilage presented significantly higher ($p < 0.05$) k values than those with MI mucilage for all the concentrations. This fact could be associated with the higher level of purity of MII, favoring a better interaction among the molecules of the polysaccharide which contributes to the increase of the viscosity of the system, such as reported in a previous work with aqueous dispersions made with the same type of mucilage (Capitani et al., 2015). The k values increased and n values decreased significantly ($p < 0.05$) as enhancing mucilage concentration from 0.25 to 1.00% wt/wt in the aqueous phase. This behavior was similar at all the tested storage times. In addition, no significant differences ($p > 0.05$) were detected for k and n values as a function of storage time, for each concentration and between the mucilages obtained by each method.

3.3. Emulsion stability

All the emulsions presented an average initial pH of 6.75 ± 0.15, not showing changes in this parameter for the different extraction methods and concentrations of chia mucilage studied.

Fig. 3 shows two typical profiles obtained for the O/W emulsions with and without the addition of 0.25 and 0.75% wt/wt chia mucilage (MI). It is worth mentioning that the emulsions prepared with the same concentrations of MII mucilage exhibited similar profiles.

A shift in the BS profiles in relation with storage time can be observed in Fig. 3a and b due to a decrease in scattered light, because of the lower concentration of droplets in Zone I of the tube. The destabilization by creaming (droplet migration to the upper portion of the tube) was indicated by a decrease in the %BS values in the low part of the tube (Zone I, 10–20 mm). In contrast, the profiles shown in Fig. 3c remained constant, without changes in relation with tube height or storage time.

At initial time (t = 0), the QuickScan profiles for Zone I did not present significant variations ($p > 0.05$) in the BS values among the different emulsions studied. The emulsion without mucilage (control) presented a BS value of 79.13%. In the case of the emulsions prepared with MI, the values for 0.25, 0.50, 0.75 and 1.00% were 79.14, 77.78, 77.20 and 77.79%, respectively, and for MII the values for the same concentrations were 78.92, 78.49, 78.39 and 78.40%, respectively.

It should be noted that previous studies indicate that chia

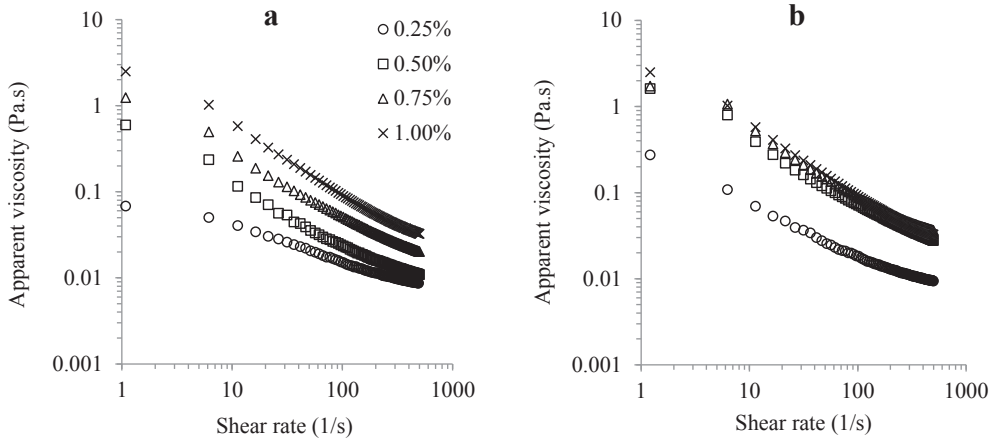

Fig. 2. Flow curves of O/W emulsions with the addition of different concentrations of chia mucilage, corresponding to the initial time: (**a**) MI and (**b**) MII.

Table 1
Power law parameters for O/W emulsions with different concentrations of chia mucilage at initial time (t = 0 d).

Concentration (% wt/wt)	k (Pa s^n)		Mean value k (Pa s^n)	n (−)		Mean value n (−)	R^2	
	MI	MII		MI	MII		MI	MII
0.00	–	–	0.001 ± 0.0001[a]	–	–	1.05 ± 0.02[d]	–	–
0.25	0.13 ± 0.06	0.24 ± 0.02	0.19[a]	0.53 ± 0.05	0.46 ± 0.02	0.49[c]	0.96	0.96
0.50	0.39 ± 0.04	1.17 ± 0.42	0.78[b]	0.42 ± 0.03	0.36 ± 0.01	0.39[b]	0.96	0.98
0.75	1.15 ± 0.02	1.89 ± 0.22	1.52[c]	0.33 ± 0.01	0.35 ± 0.02	0.34[ab]	0.98	0.95
1.00	2.76 ± 0.26	3.34 ± 0.03	3.05[d]	0.27 ± 0.01	0.34 ± 0.00	0.31[a]	0.95	0.96
Mean value	1.11[a]	1.66[b]		0.39[a]	0.38[a]			

Values followed by different letters differ significantly ($p < 0.05$) among different types and mucilage concentrations, according to Tukey's test.
Results are expressed as (x ± d), being x the average and d, the standard deviation ($n = 3$).
MI: Method I; MII: Method II.

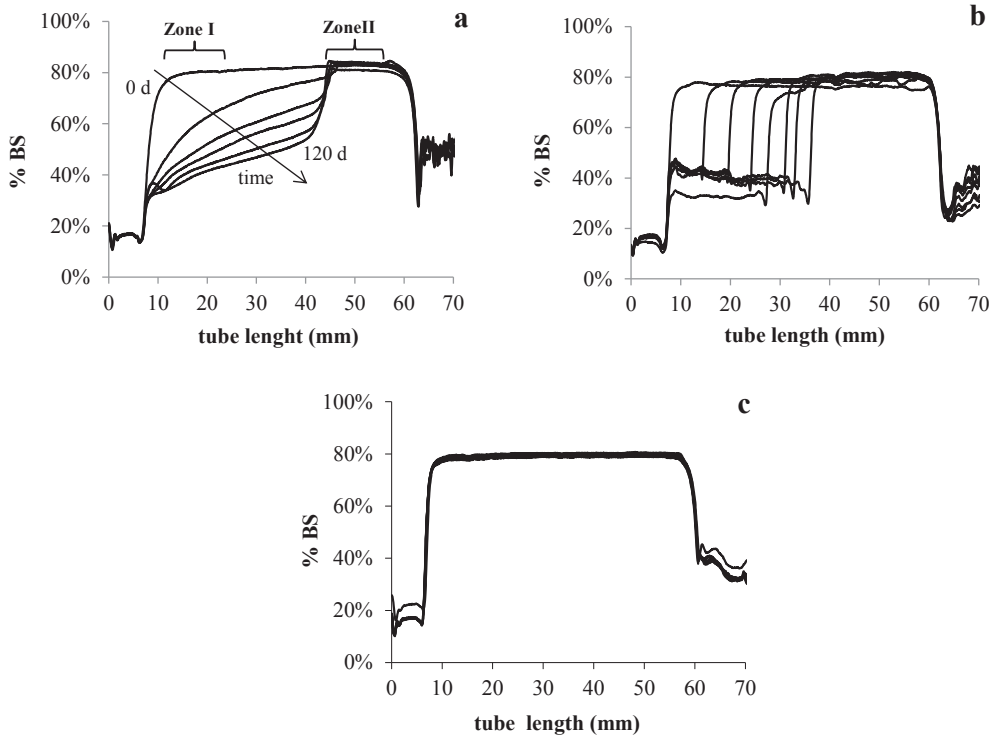

Fig. 3. Backscattering (%BS) profiles of O/W emulsions with the addition of chia mucilage (MI): (**a**) 0.00% (**b**) 0.25% and (**c**) 0.75% wt/wt.

mucilage presents emulsifying properties associated with the contribution of a low protein concentration as an integral part of the polysaccharide in the surface activity of chia mucilage dispersions, and with the stratification of the mucilage material in the vicinity of an oil-water interface (Timilsena et al., 2016; Ávila-de la Rosa et al., 2015). The results obtained in this work, with Tween 80 added in all the emulsions, suggest the need for further studies on emulsions without this emulsifying agent in order to analyze its possible interaction with the chia mucilage.

Significant differences ($p < 0.05$) were observed at 7 days of storage between the mucilages obtained by each method and at different concentrations (0.00–1.00%), without any significant interaction ($p > 0.05$) between these two variables for the BS value. The %BS of the MII emulsions were significantly higher ($p < 0.05$) than those obtained for MI emulsions, which could be attributed to the higher level of purity of MII and/or to its higher viscosity (Capitani et al., 2015). Studies carried out by Wang, Li, Wang, and Adhikari (2011) on flax mucilage and Garti et al. (1997) on fenugreek gum indicated that polysaccharides with certain protein content may improve or otherwise not affect the physical stability of O/W emulsions. However, this behavior can vary depending on the interactions among proteins and polysaccharides (Dickinson, 2003) as well as the interaction between the proteins accumulated in the continuous phase and those adsorbed at the interface (Muñoz, Alfaro, & Zapata, 2007). Moreover, the increase in viscosity of the continuous phase of the O/W emulsion reduces the mobility of the oil droplets, hindering their collision and favoring their stability (Garti & Leser, 2001; Vegi et al., 2009).

On the other hand, emulsions formulated with the lowest mucilage concentration assayed (0.00, 0.25%) were more unstable, showing a marked significant decrease ($p < 0.05$) in %BS with respect to the other concentrations tested (Fig. 4a and b). This behavior can be explained by the fact that low concentrations of polysaccharides in an emulsion favor faster rates of flocculation, coalescence and creaming (Ye, Hemar, & Singh, 2004). A similar behavior was observed in O/W emulsions formulated with 0.2% gum of *Lepidium perfoliatum* seeds (Soleimanpour et al., 2013).

Between 14 and 62 days of refrigerated storage, the emulsions with 0.00 and 0.25% mucilage continued being significantly more unstable ($p < 0.05$) than those with larger concentrations of

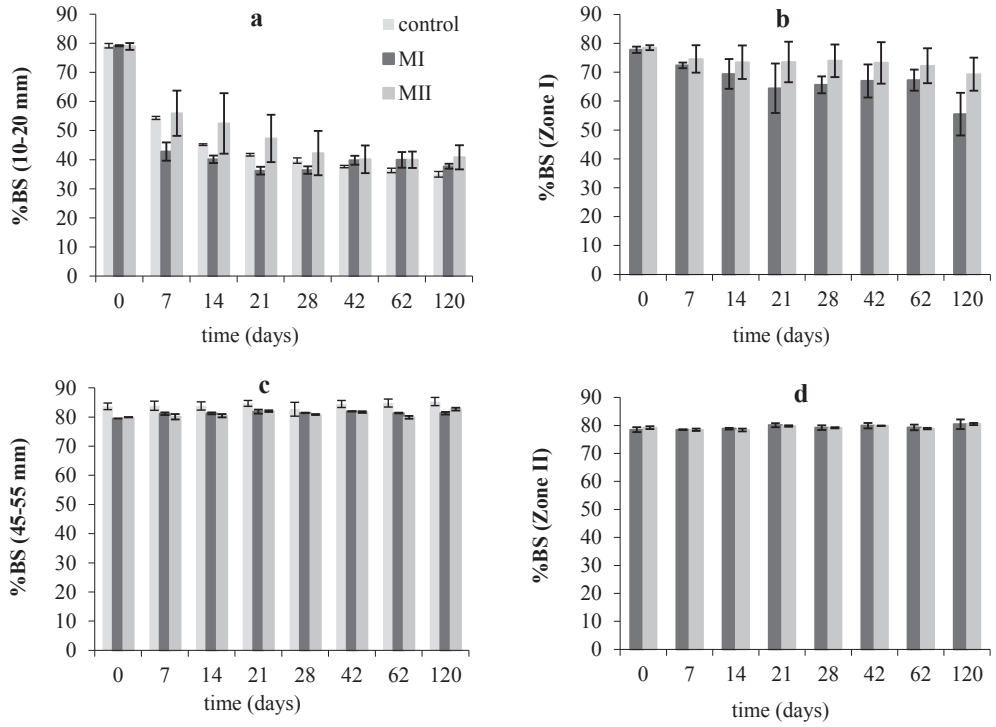

Fig. 4. Backscattering (%BS) values of O/W emulsions with the addition of different concentrations of chia mucilage in Zone I (10–20 mm): (**a**) 0.00% (control) and 0.25% (MI, MII) (**b**) 0.50% (MI, MII) and Zone II (45–55 mm): (**c**) 0.00% (control) and 0.25% (MI, MII) (**d**) 0.50% wt/wt (MI, MII). Each value is an average of two determinations ($n = 2$).

mucilage, but no significant differences were observed between mucilages ($p > 0.05$).

At the end of the storage time studied ($t = 120$ d), a significant destabilization ($p < 0.05$) of the emulsions with 0.50% chia mucilage began to be detected with respect to those with higher concentrations (>0.75%), although it was still significantly more stable ($p < 0.05$) than the emulsions with 0.00 and 0.25% mucilage (Fig. 4b). A significant difference ($p < 0.05$) was also observed at this storage time between both mucilages, similar to that detected at 7 days of storage.

It is noteworthy that for both mucilages, the O/W emulsions with higher mucilage concentration (0.75 and 1.00% wt/wt) presented a high stability during the storage time studied, without recording significant differences ($p > 0.05$) in BS values (average values: 75.31 ± 2.47 and 77.04 ± 0.68%BS for MI and 77.60 ± 0.55 and 78.05 ± 0.58%BS for MII), for 0.75 and 1.00% wt/wt, respectively. This fact could be attributed to the increase in continuous phase viscosity. Similar results were reported for O/W emulsions formulated with different concentrations of other hydrocolloids (Erçelebi & Ibanoğlu, 2010; Huang, Kakuda, & Cui, 2001; Koocheki et al., 2009a; Mahfoudhi, Chouaibi, Donsi, Ferrari, & Hamdi, 2012; Sun et al., 2007). On the other hand, when Guiotto et al. (2016) analyzed the behavior of O/W emulsions (10:90 wt/wt, oily phase sunflower-chia oil blends) formulated with different types and concentrations of modified sunflower lecithin, they observed that the addition of 0.75% chia mucilage to these emulsions contributed to a greater emulsion stability against creaming during the tested storage time (120 days). The authors attributed this fact to the decrease in the mobility of the oil droplets due to the formation of a tridimensional network, and they suggested that there is an interaction between the modified lecithins and chia mucilage that affects the consistency and particle size of these emulsions.

In the case of Zone II (45–55 mm) of the tube (characterized by the accumulation of oil droplets after the gravitational separation: cream phase), the BS values corresponding to initial time presented no significant differences ($p > 0.05$) between the mucilages obtained by each method; however the emulsion without mucilage (control) exhibited a BS value (83.67%) significantly higher ($p < 0.05$) than the rest of the concentrations analyzed (0.25–1.00%), which showed no differences between them. The BS values for the MI emulsions with 0.25, 0.50, 0.75 and 1.00% mucilage were 79.51, 78.50, 78.72 and 78.53%, respectively, and for MII the values for the same concentrations were 79.90, 79.20, 79.93 and 79.76%, respectively. For each one of the other storage times analyzed, in this Zone significant differences were observed only among concentrations ($p < 0.05$), unlike Zone I. The emulsion without mucilage presented the same behavior as for initial time compared to the emulsions with mucilage (Fig. 4c). The emulsions with 0.25% chia mucilage exhibited a slight significant increase ($p < 0.05$) of the BS value at 7 days of refrigerated storage compared to the emulsions with ≥0.50%wt/wt, and it remained constant until the end of storage time (average values: 81.47 ± 0.30 and 81.10 ± 1.05%BS for MI and MII, respectively) (Fig. 4c and d). These results indicate the formation of a stable cream phase.

It should be noted that the emulsions with ≥0.50% mucilage were not significantly different among them regarding the BS value of Zone II at all the storage times studied.

On the other hand, the emulsions formulated with 0.75 and 1.00% chia mucilage presented constant BS values over the entire period (average values: 78.58 ± 0.60 and 77.93 ± 0.61 for MI, and 79.26 ± 0.64 and 79.36 ± 0.59 for MII) for 0.75 and 1.00% wt/wt, respectively, with the results being consistent with those observed for Zone I of the tube. This behavior confirmed the positive effect of the addition of polysaccharides at these concentrations against creaming in O/W emulsions, as it was reported by other authors for the same type of emulsions (Huang et al., 2001; Koocheki et al., 2009a; Sun et at., 2007). It is worth mentioning that gums can exhibit a different protection against creaming given the

differences in molecular structure, net electric charge, hydrophilic nature and strength of the three-dimensional network of a gum in the aqueous phase, as well as the storage conditions of the emulsions (Djordjevic, Cercaci, Alamed, McClements, & Decker, 2008).

3.4. Droplet size distribution

The droplet size distribution of the emulsions at the start of their preparation (t = 0) exhibited in general a unimodal character, with droplet size ranging between 1 and 10 μm (Fig. 5a and b, MI and MII, respectively). This behavior could be attributed to the characteristics of the homogenizer used, which allows to obtain small droplet sizes (McClements, 1999). However, for emulsions with 0.75 and 1.00% of chia mucilage obtained by each method, a second population of larger droplets (10–100 μm) was detected. This fact that can be associated with an increase in the viscosity of the aqueous phase generated by a higher mucilage concentration, since previous studies have indicated that the viscosity of aqueous dispersions of chia mucilage increases with higher concentrations of mucilage (Capitani et al., 2015). In this sense, the homogenization process could be affected, preventing a complete disruption and distribution of the droplets. Similar results were observed by Koocheki et al. (2009a) when they added different concentrations of *Alyssum homolocarpum* gum to O/W emulsions prepared with the same type of homogenizer as used in this work.

At longer storage time, the formation of a population of larger droplets (100–1000 μm) was observed in emulsions formulated with lower MI mucilage concentration, reaching a maximum peak at 28 days (Fig. 5c).

At initial time (t = 0), the De Brouker mean diameters for different emulsions varied significantly ($p < 0.05$) according to the concentrations and extraction methods used, without any significant interaction ($p > 0.05$) between both factors (Table 2).

The emulsions formulated with MII exhibited significantly smaller droplet diameters ($p < 0.05$) than the MI emulsions. On the other hand, by increasing the mucilage concentration (0.00–1.00%),

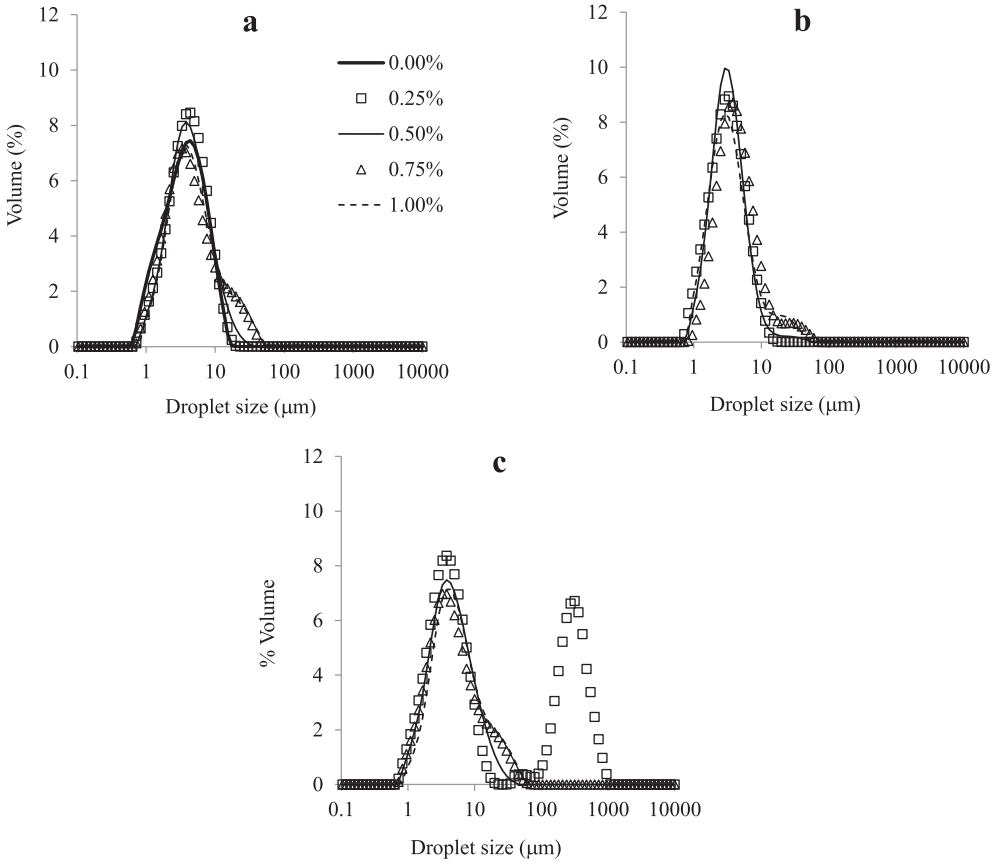

Fig. 5. Droplet size distribution of O/W emulsions with the addition of different concentrations of chia mucilage at t = 0: (**a**) control and MI (**b**) MII and (**c**) at t = 28d, MI.

Table 2
De Brouker (D[4,3]) mean diameters (μm) for O/W emulsions with the addition of different concentrations of chia mucilage, at initial time (t = 0).

Chia mucilage	Concentration (% wt/wt)					Mean value
	0.00	0.25	0.50	0.75	1.00	
MI	–	4.38 ± 0.27	4.84 ± 0.40	6.07 ± 0.26	6.53 ± 1.09	5.46^{b}
MII	–	3.42 ± 0.45	3.73 ± 1.00	5.71 ± 0.45	4.72 ± 0.29	4.40^{a}
Mean value	4.33^{ab}	3.90^{a}	4.28^{ab}	5.89^{c}	5.63^{bc}	

Values followed by different letters differ significantly ($p < 0.05$) among different types and mucilage concentrations, according to Tukey's test.
Results are expressed as (x ± d), being x the average and d, the standard deviation ($n = 3$).
MI: Method I; MII: Method II.

Table 3
De Brouker (D [4.3]) mean diameters (μm) for O/W emulsions with the addition of 0.25% (wt/wt) of chia mucilage (MI) for up to 28 days of storage.

Storage time (d)	D [4.3] (μm)
0	4.38 ± 0.27^{a}
7	7.11 ± 2.41^{ab}
14	9.51 ± 0.57^{b}
21	9.74 ± 0.55^{b}
28	14.18 ± 1.33^{c}

Values followed by different letters differ significantly ($p < 0.05$) among different as a function of storage time, according to Tukey's test.
Results are expressed as ($x \pm d$), being x the average and d, the standard deviation ($n = 3$).

in general the D[4,3] mean diameter increased significantly ($p < 0.05$), in agreement with the results observed for particle distribution.

For all the storage periods studied, the D[4,3] mean diameter values of the emulsions with MI mucilage were significantly higher ($p < 0.05$) than those with MII mucilage (average values: 6.81 ± 1.00 and 5.71 ± 0.82 μm, respectively).

Brouker mean diameters as a function of refrigerated storage time of the emulsions formulated with 0.25% mucilage (MI) for up to 28 days of storage are presented in Table 3.

The D[4,3] values of emulsions formulated with MI mucilage exhibited a significant increase ($p < 0.05$) with storage time, which would indicate that there was an insufficient amount of polysaccharide molecules to inhibit the mobility of the emulsion

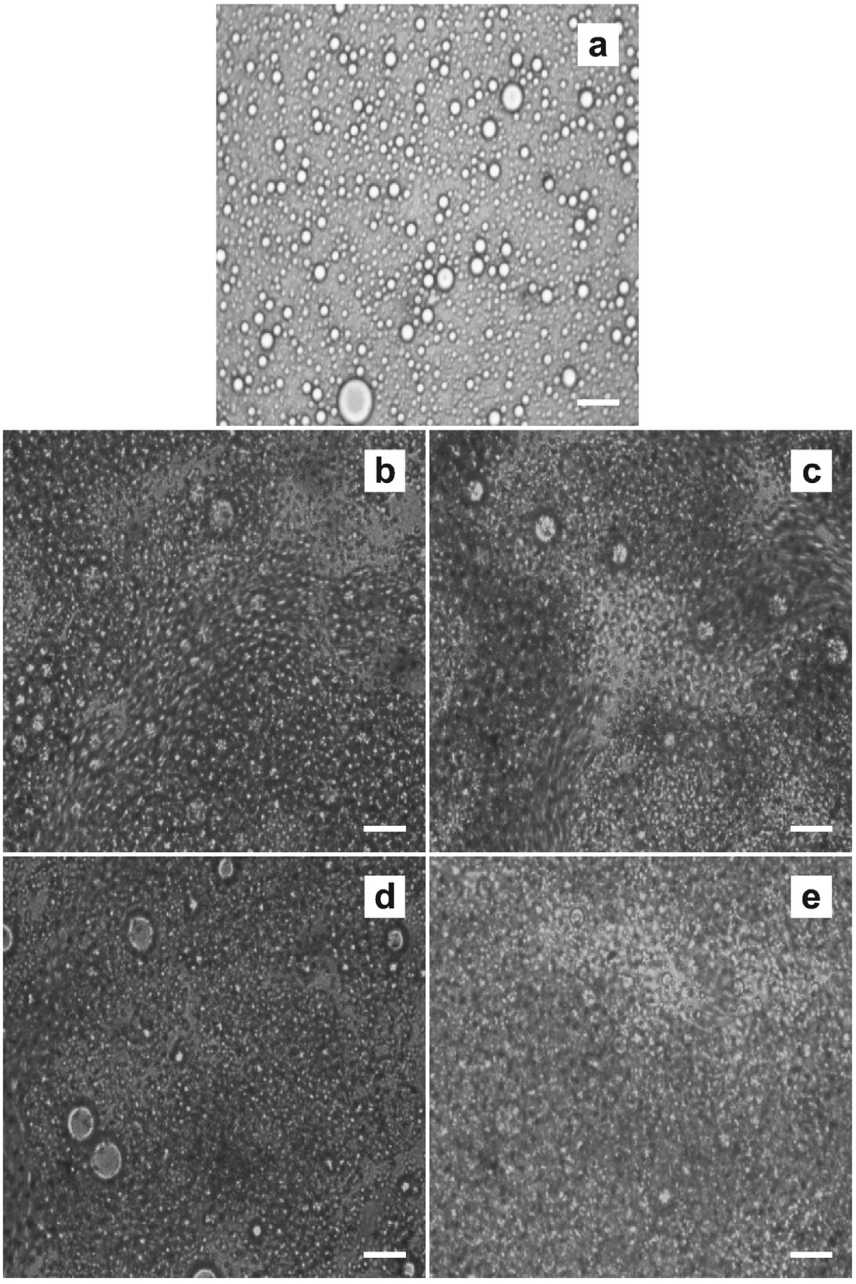

Fig. 6. Micrographs of O/W emulsions with different concentrations of chia mucilage at t = 0: (**a**) 0.00% (**b**) 0.25%, MI (**c**) 0.25%, MII (**d**) 1.00%, MI and (**e**) 1.00% wt/wt, MII. 63x, white bars: 20 μm.

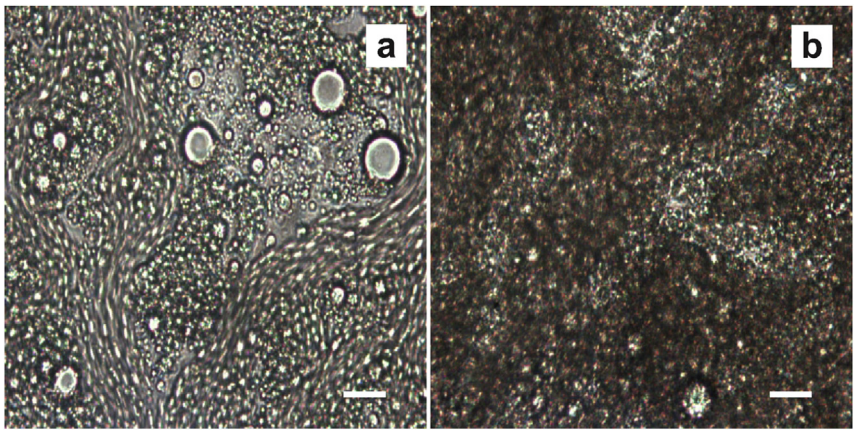

Fig. 7. Micrographs of O/W emulsions with 0.25% wt/wt chia mucilage at t = 120 d: (**a**) MI and (**b**) MII. 63x, white bars: 20 μm.

droplets and the collision frequency, and thus favor coalescence. The increase can be ascribed to the higher instability presented by this emulsion, with these results being in agreement with those obtained with the vertical analyzer (QuickScan), in which creaming increased with storage time (Fig. 3b). This behavior is also consistent with the results reported by McClements (1999), who suggested that the creaming rate is directly proportional to the size of the droplets of the emulsion. In contrast, the D[4,3] values of the emulsions formulated with 0.25% MII mucilage no exhibit significantly difference ($p > 0.05$) over the entire storage period (average value: 3.73 ± 0.14 μm).

Regarding the emulsions with ≥0.50% mucilage from both extraction methods, no statistically significant ($p > 0.05$) tendency in the variation of D[4,3] values was detected as a function of refrigerated storage time (averages values for 0.50, 0.75 and 1.00% of mucilage: 5.30 ± 0.31, 6.41 ± 0.34, 7.37 ± 0.24 μm and 4.98 ± 0.98, 7.12 ± 1.07, 7.03 ± 1.53 μm, for MI and MII respectively).

3.5. Microscopic observation

Immediately after preparation, the emulsions formulated with chia mucilage obtained by each method (MI and MII) presented a more compact appearance with increasing mucilage concentration in the aqueous phase (0.00–1.00%), thus maintaining the droplets in a stronger three-dimensional structure. This phenomenon was more evident in the emulsions with MII (Fig. 6).

Similar structures to those exhibited by emulsions with ≥0.75% wt/wt mucilage were reported by Huang et al. (2001) in O/W emulsions with 0.50% fenugreek gum.

Micrographs of the emulsions with 0.25% wt/wt MI and MII mucilage corresponding to the final stage of storage (120 d) are shown in Fig. 7. Droplets of a larger size compared with those at initial time (t = 0) can be observed, especially in the emulsions formulated with MI (Fig. 7a).

These results could be attributed to the lower aqueous phase viscosity of these emulsions, which allows a greater mobility of the oil droplets, favoring their collision with each other, and the formation of flocculi or larger droplets (coalescence). This behavior also indicates the instability of these emulsions as increasing refrigerated storage time. Similar results were reported for O/W emulsions formulated with different concentrations of *Alyssum homolocarpum* gum (Koocheki et al., 2009a).

4. Conclusions

The viscosity of the O/W emulsions formulated with chia mucilage was affected by the concentration and type of mucilage, and it was higher with increasing mucilage concentration, and no differences were observed during storage time. This parameter was higher in emulsions with MII mucilage. All the emulsions exhibited a pseudoplastic flow behavior. The addition of chia mucilage in concentrations ≥0.75% wt/wt improved the physical stability of O/W emulsions against flocculation, coalescence and the gravitational separation of phases, during all storage time. The stability of the emulsions was also affected by the extraction method used, and it was higher in the emulsions formulated with mucilage obtained by Method II (which presents a higher level of purity than MI mucilage). Based on the results obtained, chia mucilage could be used in the food industry as a thickening/stabilizing agent in the formulation of emulsified foods, such as desserts, dressings and juices.

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