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Chia seed oil-in-water emulsions as potential delivery systems of ω-3 fatty acids

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ABSTRACT

Chia oil can be incorporated into oil-in-water (O/W) emulsions as ω-3 fatty acid delivery systems in food matrices. Chia O/W emulsions varying in sodium caseinate (NaCas) concentration (2, 5 and 10% (wt/wt)), with and without 10% (wt/wt) lactose and using different homogenization pressures (400, 600 bar) were prepared, stored at 4 ± 1 °C and analyzed as a function of particle size, ζ-potential, rheological properties, backscattering profiles, peroxide and *p*-anisidine values. Droplet characteristics, rheology and stability of emulsions are influenced with different extent by the factors analyzed. All systems recorded negatively charged droplets and unimodal particle size distribution. Emulsions with 2 and 5% (wt/wt) NaCas presented a Newtonian fluid behavior. The former showed moderate stability to creaming, while the latter presented destabilization by flocculation and creaming few hours after preparation. Emulsions with 10% (wt/wt) NaCas showed a pseudoplastic fluid behavior and high stability by the rearrangement of the aggregates into a firm gel network. Chia O/W emulsions exhibited a good oxidative stability.

1. Introduction

Salvia hispanica L., commonly known as chia, is an annual plant of the *Lamiaceae* family. Although chia is an oilseed native to central and southern Mexico and Guatemala, this crop today is commercially grown in other countries as Argentina, Australia, Bolivia, Colombia, Ecuador, Nicaragua and Paraguay. The expansion of this crop and the commercialization of products that include chia seed are growing rapidly around the world, encouraged by numerous scientific papers reporting its nutritional benefits (Capitani et al., 2012; e Silva et al., 2014; Komprda et al., 2013; Luna Pizarro et al., 2013; Marineli et al., 2014; Martínez-Cruz and Paredes-López, 2014).

Chia seeds represent the vegetable source with the highest known concentration of omega-3 (ω-3) polyunsaturated fatty acids (PUFAs). Its oil contains α-linolenic acid in concentrations of up to 67.8%, which are higher than flaxseed, canola and soya oils (Gundstone and Padley, 1997). Human consumption of ω-3 PUFAs provides multiple benefits for health, protecting against heart and inflammatory disease, asthma, mood disorders and retinal diseases, along with aiding brain function; therefore, incorporating them into our diet is essential (O'Dwyer et al., 2013).

Additionally, chia seed is an excellent source of soluble fiber and antioxidants (including tocopherols, polyphenols and carotenoids), which besides being a healthy dietary and therapeutic input, serve the preservation of the oil (Ixtaina et al., 2011).

Nowadays, consumers are increasingly aware of their self-care and they seek foods fortified with health-promoting additives. Therefore, there is a growing interest in the incorporation of ω-3 fatty acids such as chia oil in functional foods. However, their incorporation may be limited due to their instability against oxidative deterioration (Ixtaina et al., 2012). The oil-in-water (O/W) emulsions represent an alternative strategic system to incorporate these rich PUFAs-oils in various food matrices and influence their oxidative stability. They can be used not only in the formulation of conventional products, such as sauces, soups, desserts,

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beverages, but also for development of powder products (microcapsules) by drying these emulsions (Hogan et al., 2001).

An O/W emulsion consists of oil droplets dispersed (disperse phase) in an aqueous medium (continuous phase) (e.g. milk, cream, beverages, and dressings) (McClements, 2010). Emulsifiers are surface-active molecules that are usually added to O/W mixtures during homogenization and form a protective membrane on surface droplet, which reduce the risk of droplet coalescence (O'Dwyer et al., 2013). Among the most widely used emulsifiers in food systems, sodium caseinate (NaCas) presents good emulsifying properties. It is composed of four principle proteins: α_{s1}, α_{s2}, β and κ caseins. It is well known that NaCas has a potential antioxidant effect by scavenging the free radical intermediates and chelating prooxidant metals (Faraji et al., 2004). Many works on the surface activity and emulsifying properties of NaCas, relates to dilute protein solutions and high oil/protein ratios. The use of NaCas as emulsifier and encapsulating agent involves drying emulsions of higher protein concentrations and lower oil phase volumes than those normally used in emulsion studies (Hogan et al., 2001).

On the other hand, lactose is a disaccharide extensively used in the food industry, recognized as the main encapsulant agent of milk fat in whole milk powder and spray-dryer dairy-like emulsions (Vega et al., 2007). Also, the addition of lactose in food protein-stabilized emulsions in order to improve its stability can exert a great influence on the emulsion properties. Several works have studied the drying of O/W emulsions formulated with NaCas and lactose, as well as their molecular assemblies (Calvo et al., 2010; Edris and Bergnståhl, 2001; Ixtaina et al., 2015; Velasco et al., 2006).

The physicochemical properties of an O/W emulsion, such as their rheology, global stability and optical properties are highly dependent on the characteristic of their droplets. In turn these characteristics are determined by the homogenization conditions (e.g. intensity and duration of energy input) and system composition (e.g. the type and concentration of emulsifier used or the viscosity ratio between disperse and continuous phases) (McClements, 2005). On the other hand, the oxidative stability of oils with a high content of ω-3 fatty acids is affected by the type, location, and concentration of proteins in emulsions (Faraji et al., 2004).

Usually, most of the research works of emulsions with oils rich in ω-3 fatty acids contain fish or flaxseed oils (Faraji et al., 2004; Fioramonti et al., 2015; Frankel et al., 2002; Horn et al., 2011; Kuhn and Cunha, 2012). However, a little information is available on chia O/W emulsions (Carrillo Navas et al., 2011).

The aim of this work was to study the incorporation of chia oil into functional O/W emulsions and to evaluate the effect of NaCas concentration, the addition of lactose and the homogenizer pressure level on its physicochemical properties.

2. Materials and methods

2.1. Materials

Commercial chia oil was provided by SDA S.A. (Lobos, Argentina) and stored until use at 4 ± 1 °C in amber glass bottles without head space. Fatty acid composition was analyzed by GC according to the IUPAC 2.302 (IUPAC, 1992) standard method as following: (C16:0) 8.5 ± 0.9%, (C18:0) 2.0 ± 0.6%, (C18:1) 5.2 ± 0.6%, (C18:2) 19.0 ± 0.6% and (C18:3) 65.4 ± 1.4%. Oil tocopherol content was determined by normal phase HPLC following the procedure described in AOCS Ce8-89 (AOCS, 1998). The tocopherol content was 443 ± 4, 6 ± 1, 415 ± 5 and 22 ± 1 (mg of tocopherol/kg oil) for total, α, γ and δ fraction, respectively. The initial peroxide, iodine, saponification and free fatty acids values were 2.0 ± 0.1 meq peroxide/kg oil, 213.0 ± 3.4 g I_2/100 g oil, 193.5 ± 0.1 mg KOH/g oil and 0.6 ± 0.1% of oleic acid, respectively. Casein sodium salt (NaCas) from bovine milk was purchased from Sigma Chemical Company (St. Louis, MO) and D-lactose monohydrate from Anedra (Argentina). All reagents used were of analytical grade.

2.2. Preparation of emulsions

Chia O/W emulsions, were constituted by an oil to water ratio of 10:90. The aqueous phase was composed of different NaCas content (2, 5 and 10% wt/wt) as emulsifying agent and lactose (0 and 10% wt/wt), while 10% (wt/wt) of chia oil constituted the oil phase. Prior to emulsification, the NaCas powder was dissolved in distilled water at 50 °C through constant mechanical stirring for 3 h to ensure a complete dissolution and then stored overnight at 4 ± 1 °C. For emulsions containing lactose, it was added to the aqueous phase while stirring at 25 °C. Emulsions were obtained through two homogenization steps. A primary homogenization was conducted using an Ultraturrax T-25 (Janke & Kunkel GmbH, Staufen, Germany) operated for 1 min at 9500 rpm, followed by a second stage using a valve high pressure homogenizer (Panda 2K, GEA NiroSoavi, Parma, Italy) at two different pressures (400 and 600 bar) and 4 passes. Nisine (0.0012 g/100 g) and potassium sorbate (0.1 g/100 g) both food grade additives, were added to the emulsions in order to prevent microbial growth.

2.3. Characterization of emulsions

2.3.1. Particle size

The particle size distribution was determined by laser diffraction with a particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, UK) in a range of measurement from 0.1 to 1000 μm according to Cabezas et al. (2012). Approximately 1 mL of emulsion was suspended directly in a water bath of the dispersion system with a pump speed of 2000 rpm (Hydro 2000MU), reaching an obscuration of 15–18%. The refractive indices of sunflower oil (1.47) as particle, and water (1.33) as dispersant, were used. Results were given in particle size distribution and De Brouker (D [4, 3]) mean diameter.

2.3.2. ζ-potential and pH

The pH was measured mixing emulsion and distilled water in a 1:1 ratio during stirring with a pH meter (Hanna Instruments, Woonsocket, USA) at room temperature.

The ζ-potential was determined using a Zeta Potential Analyzer (Brookhaven 90Plus/Bi-MAS, USA) instrument on electrophoretic mobility function at room temperature. The ζ-potential range was set from −100 to 50 mV and the electrophoretic mobility was converted into ζ-potential values using the Smoluchowski equation. For each determination 0.05 g of the emulsion was dispersed in 100 mL of milli-Q water before measurements were taken.

2.3.3. Rheological properties

Rheological measurements were carried out at 25 ± 0.3 °C with a Haake RS600 controlled stress oscillatory rheometer (Haake, Germany) using a plate–plate sensor system with a 1.0 mm gap between plates. Emulsion viscosity was determined over a shear rate ($\dot{\gamma}$) range of 1.0–500 s^{-1}. The shear stress (σ) data were then analyzed according to the power law equation $\sigma = K\dot{\gamma}^n$ calculating the consistency coefficient (K) and the flow behavior index (n).

2.3.4. Emulsion stability

Global stability of emulsions was determined by measurements of dispersed light with a Quick Scan Vertical Scan Analyzer (Coulter Corp., Miami, USA) according to Pan et al. (2002). The emulsion was transferred to a cylindrical glass tube for measurement. The entire length of the sample (about 65 mm) was scanned by a reading head composed of a pulsed near infrared light source ($\lambda = 850$ nm) and two synchronous detectors: a transmission (at 0° from the incident beam) and a backscattering detector (at 135° from the incident beam), acquiring transmission and backscattering data every 40 μm. Profiles recorded for each sample were then analyzed and the difference of creaming index (ΔCI) was determined according to Eq. (1):

$$\Delta\mathrm{CI}\,(\%) = \mathrm{CI}_t - \mathrm{CI}_0 \tag{1}$$

where

CI_t is the creaming index at different storage time ($t = 10$, 20 and 30 d),
CI_0 is the creaming index at the initial time
$\mathrm{CI}\ (\%) = (H/H_0) \times 100$,
H is the height of the serum layer formed at the bottom of glass tubes,
H_0 represents the initial height of the emulsion.

2.3.5. Peroxide value

Peroxide value was evaluated according to Díaz et al.'s (2003) method. Lipid hydroperoxides were extracted by mixing 0.3 mL of emulsion with 1.5 mL of isooctane/2-propanol (3:1, v/v) using a vortex (10 s, 3 times), and centrifuging at 3400*g* (Rolco CM 2036, Argentina) for 2 min. The isolated organic phase (0.2 mL) was added to 2.8 mL of methanol/butanol (2:1, v/v) followed by 30 μL of a thiocyanate/ferrous solution (freshly prepared by mixing equal volumes of 0.144 M $FeSO_4$ and 0.132 M $BaCl_2$, centrifuging 3 min, and then mixing equal volumes of this clear ferrous solution and 3.94 M ammonium thiocyanate). The absorbance of the solution was measured 20 min after the addition of iron at 510 nm. The concentration of lipid hydroperoxides was calculated using a cumene hydroperoxide standard curve.

2.3.6. *p*-anisidine value (*p*-An)

The *p*-An test was performed by Atarés et al.'s (2012) method. An aliquot of 1 mL of emulsion was added to a 25 mL falcon tube and filled up to the mark with isooctane. The tubes were vortexed twice for 20 s each. After centrifugation for 10 min at 5000*g* (Rolco CM 2036, Argentina), the absorbance (A_1) of the samples at 350 nm was measured against a white pure isooctane. Then, 5 mL of the remaining solution from centrifugation were transferred to test tubes of 10 mL and 1 mL of *p*-anisidine solution (0.25% solution wt/v in glacial acetic acid) was added. After vortexing for 10 s and resting for 10 min, the absorbance (A_2) was measured at 350 nm against the blank of isooctane with *p*-anisidine

$$p\text{-An} = 25(1.2 \times A_2 - A_1)/m$$

where m is the mass of the sample.

2.4. Storage of emulsions

Emulsions obtained were stored at 4 ± 1 °C and protected from light for 30 days. The physicochemical stability was evaluated periodically during storage time determining the D [4, 3] diameter, the backscattering profiles, the peroxide and *p*-anisidine values as previously described in Sections 2.3.1, 2.3.4, 2.3.5 and 2.3.6, respectively.

2.5. Experimental design and statistical analysis

A 3 × 2 × 2 fully factorial design, replicated twice, was used to study the effects of sodium caseinate concentration (2, 5 and 10% wt/wt), presence of lactose (0 and 10% wt/wt) and homogenization pressure (400 and 600 bar) on each variable studied (particle size distribution, mean diameter, ζ-potential, rheological properties, backscattering profiles and creaming index, peroxide and *p*-anisidine values). The experimental design and the sample codes are shown in Table 1. Results were analyzed by a multifactorial ANOVA test (95% level of confidence) to study the main effects and the interactions between the factors. Statistical analysis was performed using the Statgraphics Centurion software (Version XV.II for Windows, Manugistics Inc., USA). Means were separated according to Tukey's High Meaningful Difference test (95% level of confidence).

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

3. Results and discussion

The results of the multifactorial ANOVA are presented in Table 2. As can be seen the NaCas concentration was the main factor that affect the most of the physicochemical properties studied, except D [4, 3], which was influenced mainly by the homogenization pressure.

3.1. Particle size

The droplets characteristics of food emulsions such as droplet size and charge are some of the key parameters influencing physicochemical and sensory properties (McClements, 2010).

The initial D [4, 3] mean diameters are presented in Fig. 1. The results showed that the homogenization pressure and NaCas concentration had a very significant effect ($p \leqslant 0.001$) on D [4, 3], whereas the addition of lactose also affected this parameter but in lesser extent ($p \leqslant 0.05$). No interactions between factors were recorded (Table 2). A significant ($p \leqslant 0.05$) reduction of droplet size was observed at constant NaCas concentration when the homogenizer pressure was increased from 400 to 600 bar (Fig. 1). These results are in agreement with McClements (2004), who found that the oil droplet size of O/W emulsions, stabilized with an excess of emulsifier, depended mainly on the energy input of the homogenizer with a minor influence from the emulsifier concentration. Also, other authors reported that the droplet size decreased as energy of the homogenizer was increased (O'Dwyer et al., 2013; Atarés et al., 2012; Horn et al., 2012; Lethuaut et al., 2002). No significant differences ($p > 0.05$) on particle size between emulsions with 2 and 5% (wt/wt) NaCas were recorded, but the increase of emulsifier level up to 10% (wt/wt) led to a significant enhancement ($p \leqslant 0.05$) of this parameter. This fact may be due to the increase of the viscosity of these systems, which may suppress the formation of eddies responsible for breaking up droplets during the homogenization process (Qian and McClements, 2011; McClements and Weiss, 2005). In this sense, a significant positive correlation was found between D [4, 3] and viscosity of emulsions ($r = 0.47$; $p = 0.0204$). The addition of lactose promoted a decrease of D [4, 3] diameter. The volume-weighted particle size distributions from the different emulsions are shown in Fig. 2. Emulsion composition and homogenization conditions investigated in this research work recorded similar unimodal particle size distributions for all systems with a slight shift of the distribution curves toward larger droplet sizes in emulsions stabilized with 10% (wt/wt) NaCas.

Table 1
Formulations for chia O/W emulsions based on 3 × 2 × 2 full factorial design. Experimental parameters and samples codes.

Sodium caseinate concentration (%)	Lactose concentration (%)			
	0 Homogenization pressure (bar)		10 Homogenization pressure (bar)	
	400	600	400	600
2	2 NaCas_400	2 NaCas_600	2 NaCas_Lac_400	2 NaCas_Lac_600
5	5 NaCas_400	5 NaCas_600	5 NaCas_Lac_400	5 NaCas_Lac_600
10	10 NaCas_400	10 NaCas_600	10 NaCas_Lac_400	10 NaCas_Lac_600

Table 2
Multifactorial analysis of variance (ANOVA) of fully factorial design (3 × 2 × 2) for the physicochemical properties of chia O/W emulsions.

Factor	Degrees of freedom	Sum of squares							
		D [4, 3]	ζ–potential	n	K	η_{100}	BS_{av0}	PV	p-An
NaCas concentration (A)	2	0.0589***	690.2460***	0.3080***	1.1089***	0.0956***	184.6960***	6.1805***	0.0024
Lactose addition (B)	1	0.0062*	403.6780***	0.0199***	0.4026***	0.0287***	20.7018***	0.0469	0.0013
Homogenization pressure (C)	1	0.0792***	225.3120***	0.0003	0.1145***	0.0024***	4.4462	0.0826	0.0009
A × B	2	0.0023	50.4981*	0.0086***	0.7333***	0.0510***	5.2344	0.0592	0.0010
A × C	2	0.0048	166.1050***	0.0104***	0.2157***	0.0047***	18.3983**	0.0752	0.0004
B × C	1	0.0023	0.8568	0.0126***	0.1243***	0.0027***	0.0301	0.0886	0.0006
A × B × C	2	0.0017	42.4508*	0.0058***	0.2293***	0.0051***	3.9491	0.0056	0.0036
Pure error	12	0.0104	269.5150	0.0061	0.0034	0.0003	11.7076	0.7303	0.0070
Total	23	0.1659	1848.6617	0.3715	2.9320	0.1904	249.1635	7.2688	0.0172

D [4, 3] average oil droplet diameters (μm); n flow behavior index; K consistency coefficient (Pa s^n); η_{100} apparent viscosity at 100 s^{-1}; BS_{av0} initial average backscattering value (%); PV peroxide value (meq hydroperoxide/kg oil); p-An p-anisidine value.

* $p \leqslant 0.05$.

** $p \leqslant 0.01$.

*** $p \leqslant 0.001$.

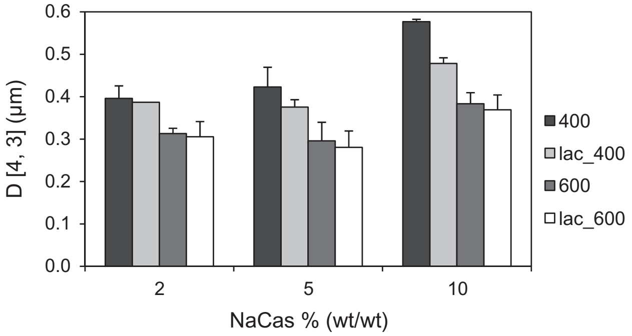

Fig. 1. De Brouker (D [4, 3]) mean diameter for chia O/W emulsions as a function of NaCas content (2, 5 and 10% wt/wt), in presence or absence of lactose, at different homogenization conditions (400 and 600 bar).

The evolution of particle size of chia O/W emulsions (D [4, 3]) during the storage period studied did not present significant changes.

3.2. ζ-potential and pH

On the other hand, the pH of chia O/W emulsions, was 6.3–6.5 and the droplet charge was strongly negative for all systems studied, ranging from −54 to −35 mV. This charge was related to the ionized groups from the NaCas layer that surrounded them, when the pH was above the isoelectric point (pI) (Hu et al., 2003). The relative importance of the main factors studied on ζ-potential is given by the following order: NaCas concentration (A) > lactose addition (B) > homogenization pressure (C) according to ANOVA. Besides, the second-level (A × B and A × C) and third-level interactions were also significant (Table 2). A positive significant correlation ($r = 0.46$; $p = 0.0226$) was found between ζ-potential and D [4, 3] diameter. A possible explanation for this association could be related to the amount of the protein adsorbed at the interface. Thus, emulsions with smaller particle size and a high surface area presented the most of protein adsorbed at the interface, while those with higher D [4, 3] had greater non-adsorbed protein content. This fact probably promotes the electrostatic association between polypeptide chains leading to the formation of multilayers and the charge suppression.

3.3. Rheological properties

The flow curves data for the different systems studied were fitted to the power law model equation (correlation coefficients > 0.99) and the values for the apparent viscosity at a shear rate of 100 s^{-1} were calculated (Table 3). This parameter was evaluated at this shear rate since it is typical of food processes, such as flow through a pipe, stirring or mastication (McClements, 2005).

NaCas concentration and lactose addition produced a highly significant influence on rheological properties of chia O/W emulsions while the homogenizer pressure level affected K and η_{100}. Second and third level interactions were significant for all rheological parameters and factors (see Table 2). In general, an enhancement of viscosity and changes of the flow properties as a function of the increasing NaCas concentration were observed (Fig. 3a, Table 3). This fact could be attributed to an increase in solid content corresponding to the non-adsorbed NaCas in the aqueous phase, which tends to a more resistance to flow, and thus, the viscosity of the emulsion system lead to an inter-droplet structure formation. On the other hand, the addition of lactose produced an increase of viscosity mainly for chia O/W emulsions at 10% (wt/wt) NaCas as can be seen in Fig. 3b.

It was observed that chia O/W emulsions with 2 and 5% (wt/wt) NaCas showed a low viscosity and behaved like Newtonian fluids probably due to the low excess of non-adsorbed protein in the

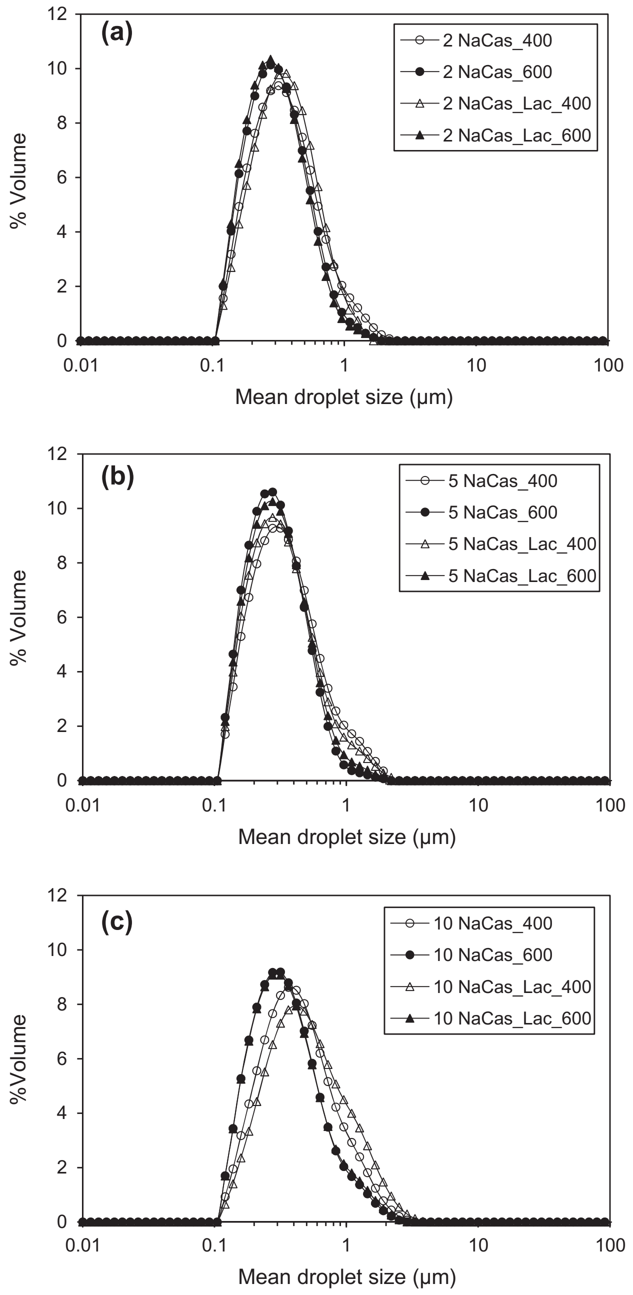

Fig. 2. Particle size distributions (% volume) of chia O/W emulsions with different NaCas concentration: (a) 2% (b) 5% and (c) 10% wt/wt, in presence or absence of lactose, at two homogenization pressure levels (400 and 600 bar). Average values are shown ($n = 2$). The coefficient of variation was lower than 1%.

aqueous phase. At 10% (wt/wt) NaCas, emulsions presented a high viscosity, which increased significantly ($p \leqslant 0.05$) with the addition of lactose into the aqueous phase. These systems presented different degrees of shear-thinning behavior as a result of a gradual network formation which could be related to the increase of the non-adsorbed protein level (Fig. 3b, Table 3). The pseudoplasticity of chia O/W emulsions with 10% NaCas (wt/wt) was associated with the mechanical disruption of the structure network (aqueous phase trapped within the interdroplet structure) at large applied stresses. These results are in agreement with those reported by Dickinson and Golding (1997) and Huck-Iriart et al. (2011); for NaCas stabilized emulsions which presented a highly dependent on the concentration of NaCas, with a Newtonian behavior for systems with low concentration of free protein in the bulk and shear-thinning at high NaCas content.

3.4. Emulsion stability

In terms of global stability, backscattering profiles (%BS) of the different O/W emulsion systems were performed as a function of length of the measuring tube (cm) at different storage times. The evolution of BS profiles of chia O/W emulsions with different NaCas content and two levels of homogenization pressure are shown in Fig. 4. Both emulsions with 2 and 10% (wt/wt) NaCas presented a high global stability, maintaining BS profiles without major changes during the period studied. In contrast, emulsions with 5% (wt/wt) NaCas exhibited a poor stability, recording a destabilization immediately after preparation. The average values of initial BS along the entire tube (BS_{av0}, from BS profile at $t = 0$) were 68.77, 61.78 and 64.68% for systems with 2, 5 and 10% (wt/wt) NaCas, respectively. Taking into account that BS is a parameter directly related with the droplet size (Álvarez Cerimedo et al., 2010), these values were similar to D [4, 3] with the exception of emulsions with 5% (wt/wt) of emulsifier. In this case, the low mean BS value recorded could be associated with the formation of flocs.

The statistical analysis evidenced a high influence of NaCas concentration and lactose addition and a second-level interaction between NaCas concentration and homogenization pressure on emulsion stability (see Table 2). Thus, when emulsions were stabilized with 2% (wt/wt) NaCas, slight signs of destabilization by creaming were evidenced through a decrease in %BS at the bottom of the tube and with a simultaneous increase of %BS in the top, due to the migration of individual oil droplets to the upper zone (Fig. 4a). These systems presented droplets with smaller particle size than 10% (wt/wt) NaCas. However, these characteristics did not avoid to record slight changes in the BS profiles which could be associated with the onset of the creaming process.

For emulsions with 5% (wt/wt) NaCas the destabilization occurred through both flocculation and creaming mechanisms. In this case, the BS% decreased in the bottom and the middle of the tube due to the formation of aggregates with a sharp increase of this parameter in the upper zone corresponding to the creaming of these flocs (Fig. 4b). Depletion flocculation was probably promoted by non-adsorbed biopolymers in the aqueous phase, leading to an increase in the attractive force between the droplets until it eventually become large enough to overcome the repulsive interactions between the droplets and cause them to flocculate (McClements, 2004). The creaming of these flocculated emulsions was fast and marked which could be due to the large size of these aggregates and/or flocs. In case of emulsions with 10% (wt/wt)

Table 3
Rheological parameters (n, flow behavior index; K, consistency coefficient) obtained from the power law model and the apparent viscosity at 100 s^{-1} (η_{100}) for the chia O/W emulsions measured at 25 ± 0.3 °C.

System	n	K (Pa s^n)	η_{100} (Pa s^n) × 10^{-3}
2 NaCas_400	1.01 ± 0.01^{cd}	0.003 ± 0.001^{a}	2.8 ± 0.2^{a}
2 NaCas_600	1.01 ± 0.02^{cd}	0.002 ± 0.001^{a}	2.6 ± 0.1^{a}
2 NaCas_lac_400	1.01 ± 0.01^{cd}	0.003 ± 0.000^{a}	3.3 ± 0.2^{a}
2 NaCas_lac_600	1.0 ± 0.01^{c}	0.003 ± 0.001^{a}	3.3 ± 0.4^{a}
5 NaCas_400	0.99 ± 0.04^{c}	0.006 ± 0.002^{a}	5.5 ± 0.9^{a}
5 NaCas_600	1.07 ± 0.01^{d}	0.004 ± 0.000^{a}	5.0 ± 0.7^{a}
5 NaCas_lac_400	0.97 ± 0.01^{c}	0.009 ± 0.001^{a}	7.7 ± 0.2^{a}
5 NaCas_lac_600	0.96 ± 0.01^{c}	0.008 ± 0.000^{a}	6.8 ± 0.3^{a}
10 NaCas_400	0.82 ± 0.01^{b}	0.085 ± 0.01^{b}	37.0 ± 3.3^{b}
10 NaCas_600	0.84 ± 0.02^{b}	0.073 ± 0.01^{b}	34.6 ± 2.3^{b}
10 NaCas_lac_400	0.81 ± 0.0^{b}	0.375 ± 0.021^{c}	156.3 ± 8.8^{c}
10 NaCas_lac_600	0.69 ± 0.01^{a}	1.095 ± 0.049^{d}	262.7 ± 11.8^{d}

Different letters in the same column indicate significant differences ($p \leqslant 0.05$).

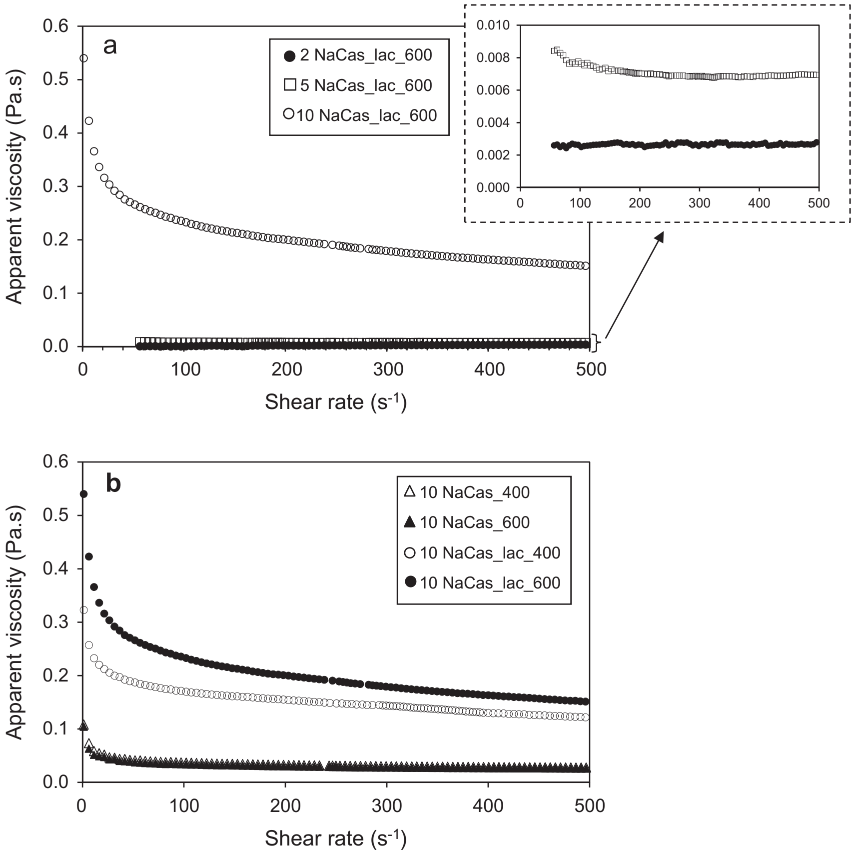

Fig. 3. Effect of different formulations and homogenization pressure on rheological properties of chia O/W emulsions. Viscosity–stress curves of chia O/W emulsions stabilized with (a) 2, 5 and 10% (wt/wt) of NaCas, (b) 10% NaCas with or without lactose and homogenized at 400 and 600 bar. Average values are shown ($n = 3$). The coefficient of variation was lower than 1%.

NaCas, these systems remained fairly constant in terms of their BS profiles, showing only a slight decrease during storage which could be associated with a slow flocculation (Fig. 4c). This high global stability could be attributed to the restabilization of the emulsion in the form of a strong particle gel network and their high viscosity, which hinders the migration of droplets to the top of the tube (Huck-Iriart et al., 2011). Similar trends were observed by Dickinson et al. (1997).

In addition, the ΔCI% of emulsions were calculated at different storage time. The statistical analysis revealed that NaCas concentration represented the main significant factor ($p \leqslant 0.01$) that affects this index related to the emulsion stability. Fig. 5 shows that 5% (wt/wt) NaCas emulsions presented the most increase of this parameter in comparison with the other ones. In contrast, 10% (wt/wt) NaCas emulsions were stable against this destabilization process during the period studied ($p > 0.05$). These facts are in accordance to the experimental results described previously. A negative statistical correlation ($p \leqslant 0.05$) was found between ΔCI% at the different times and viscosity (η_{100}) ($r = -0.43$; -0.45; -0.56 for $\Delta CI\%_{10d}$ $\Delta CI\%_{20d}$ and $\Delta CI\%_{30d}$, respectively). Thus, an increase in the viscosity produces a decrease in the particle mobility, resulting in an enhancement of the emulsion stability.

3.5. Peroxide and p-An values

Regarding the oxidative stability of chia O/W emulsions, the only highly significant influence on PV values was NaCas concentration (see Table 2). The initial level of lipid hydroperoxides in emulsions was low, indicating that the emulsification process did not contribute to increase the hydroperoxide content. Emulsions formulated with 10% (wt/wt) NaCas presented significantly ($p \leqslant 0.05$) higher PV than the other ones which could be associated with its high particle size. This fact is in agreement with the positive significant correlation ($r = 0.58$; $p = 0.0029$) found between D [4, 3] and PV. In literature there is no a clear trend on the lipid oxidation and its relation to the particle size measurements. Previous studies reported results consistent with those presented in this work, where the decreasing particle size in fish oil O/W emulsions decreased lipid oxidation (Nakaya et al., 2005; Let et al., 2007). However, other works indicated an increase in the rate of lipid oxidation when droplet diameter decreased and explained it in terms of an increase in droplet surface and the possibility of an attack by oxidation inducers such as free radicals or metal ions on the lipids at the interface (Atarés et al., 2012).

The evolution of PV values for chia O/W emulsions with different NaCas content stored at 4 ± 1 °C, 30 d can be seen in Fig. 6. At the end of storage, PV values were 8.15, 4.50, 2.00 and 1.80 meq hydroperoxide/kg oil for chia oil and emulsions with 10, 2 and 5% (wt/wt) NaCas, respectively. Chia bulk oil recorded significantly higher ($p \leqslant 0.05$) peroxide values than O/W emulsions from the first week of the refrigerated storage. All systems exhibited lower levels of primary oxidation by-products than the upper limit (10 meq hydroperoxide/kg oil) stablished by the *Codex Alimentarius* (Codex Alimentarius Commission, 2012) for human consumption of oils not covered by individual standards.

In the case of secondary oxidation products monitored through measurements of *p*-An values no significant effects of the different

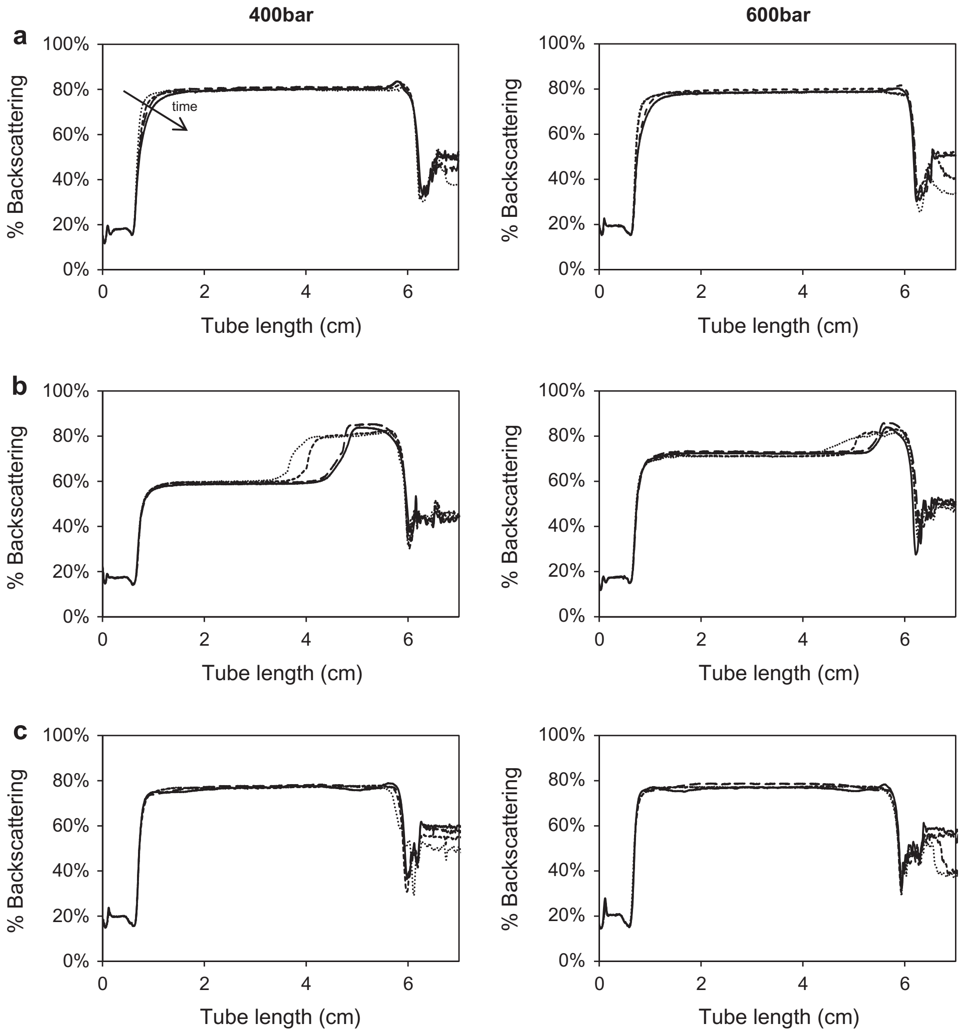

Fig. 4. Backscattering profiles (%BS vs. tube length) of chia O/W emulsions prepared with (a) 2% (b) 5% and (c) 10% of NaCas as a function of time (••••••••) 20 h, (▬ ▬ ▬) 7, (▬▬ ▬▬) 20 and (▬▬▬) 30 d of storage at two levels of homogenization pressure (400 and 600 bar). Average values ($n = 2$).

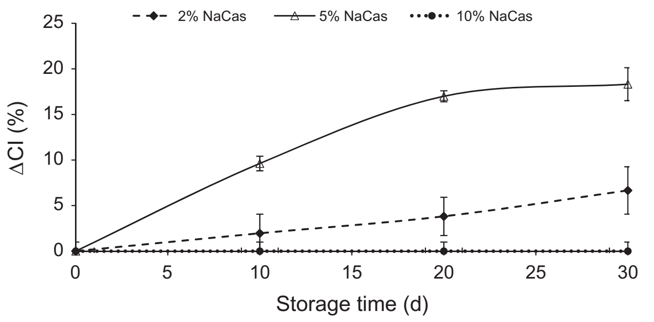

Fig. 5. Evolution of the difference of creaming index (ΔCI (%)) of chia O/W emulsions as a function of storage time. Average values ($n = 2$).

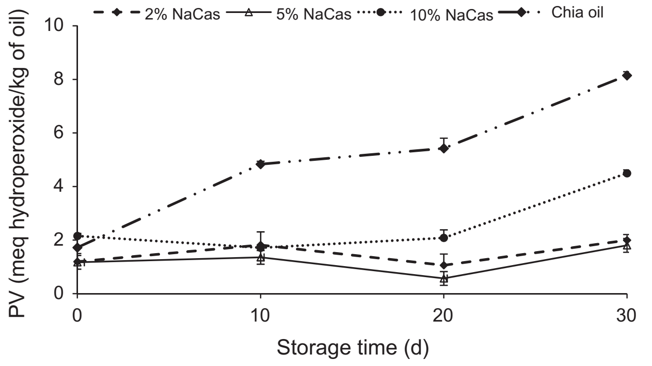

Fig. 6. Peroxide value of chia O/W emulsions with different NaCas content at constant homogenizer pressure of 600 bar and chia bulk oil. Average values ($n = 2$).

factors investigated in the experimental design were recorded, presenting low levels in all cases (p-An < 1).

These results suggest that emulsions could be a suitable system, which contribute to increase the oxidative stability of oils for providing additional protection such as the presence of a droplet membrane, interaction between ingredients, and partitioning of ingredients between the oil (O'Dwyer et al., 2013).

4. Conclusions

Chia O/W emulsions were investigated in order to evaluate the influence of sodium caseinate concentration, the presence of lactose and the homogenizer pressure level on their physicochemical properties. These factors were very important because affected the most characteristics studied.

Chia O/W emulsions presented negatively charged droplets (pH 6.4) with unimodal droplet size distribution being the droplet size highly dependent on the homogenizer pressure, sodium caseinate concentration and in a lesser extent of the addition of lactose.

The global stability and rheological properties of chia O/W emulsions were mainly influenced by sodium caseinate content and lactose addition. A moderate stability and Newtonian behavior was exhibited by the chia O/W emulsions stabilized with 2% (wt/wt) of emulsifier. At 5% (wt/wt) of sodium caseinate, depletion flocculation was evidenced and emulsions presented a poor stability as a consequence of the increase of non-adsorbed emulsifier and the formation of aggregates. Emulsions formulated with 10% (wt/wt) of sodium caseinate exhibited a pseudoplastic fluid behavior and showed a high stability by the rearrangement of the aggregates into a firm gel network.

Chia O/W emulsions recorded low levels of primary and secondary oxidation products during the storage period studied. Chia bulk oil exhibited higher levels of PV and *p*-An than emulsified oil.

The results obtained could suggest that O/W emulsions are a potential alternative system to improve chia oil stability against lipid oxidation and at the same time incorporate them into diverse functional foods.

Conflict of interest

The authors declare no conflict of interest.

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References

Álvarez Cerimedo, M.S., Iriart, C.H., Candal, R.J., Herrera, M.L., 2010. Stability of emulsions formulated with high concentrations of sodium caseinate and trehalose. Food Res. Int. 43 (5), 1482–1493.

AOCS, 1998. Official Methods and Practices of the AOCS, fifth ed. AOCS Press, Champaign, USA.

Atarés, L., Marshall, L.J., Akhtar, M., Murray, B.S., 2012. Structure and oxidative stability of oil in water emulsions as affected by rutin and homogenization procedure. Food Chem. 134 (3), 1418–1424.

Cabezas, D., Madoery, R., Diehl, B., Tomás, M., 2012. Emulsifying properties of different modified sunflower lecithins. J. Am. Oil. Chem. Soc. 89 (2), 355–361.

Calvo, P., Hernández, T., Lozano, M., González-Gómez, D., 2010. Microencapsulation of extra-virgin olive oil by spray-drying: influence of wall material and olive quality. Eur. J. Lipid Sci. Technol. 112 (8), 852–858.

Capitani, M.I., Spotorno, V., Nolasco, S.M., Tomás, M.C., 2012. Physicochemical and functional characterization of by-products from chia (*Salvia hispanica* L.) seeds of Argentina. LWT – Food Sci. Technol. 45 (1), 94–102.

Carrillo Navas, H., Cruz-Olivares, J., Varela-Guerrero, V., Alamilla-Beltrán, L., Vernon-Carter, E.J., Pérez-Alonso, C., 2011. Rheological properties of a double emulsion nutraceutical system incorporating chia essential oil and ascorbic acid stabilized by carbohydrate polymer–protein blends. Carbohydr. Polym. 87, 1231–1235.

Codex Alimentarius Commission. Codex Stan 19. Edible fats and oils not covered by individual standards. <http://www.codex alimentarius.net/web/standard_list.do> (access August 2012).

Díaz, M., Dunn, C.M., McClements, D.J., Decker, E.A., 2003. Use of caseinophosphopeptides as natural antioxidants in oil-in-water emulsions. J. Agric. Food Chem. 51 (8), 2365–2370.

Dickinson, E., Golding, M., 1997. Rheology of sodium caseinate stabilized oil-in-water emulsions. J. Colloid Interface Sci. 191 (1), 166–176.

Dickinson, E., Golding, M., Povey, M.J., 1997. Creaming and flocculation of oil-in-water emulsions containing sodium caseinate. J. Colloid Interface Sci. 185 (2), 515–529.

e Silva, B.C., dos Santos, H.M.C., Montanher, P.F., Boeing, J.S., Almeida, V., Visentainer, J.V., 2014. Incorporation of omega-3 fatty acids in Nile tilapia (*Oreochromis niloticus*) fed chia (*Salvia hispanica* L.) bran. J. Am. Oil. Chem. Soc. 91 (3), 429–437.

Edris, A., Bergnståhl, B., 2001. Encapsulation of orange oil in a spray dried double emulsion. Food/Nahrung 45 (2), 133–137.

Faraji, H., McClements, D.J., Decker, E.A., 2004. Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. J. Agric. Food Chem. 52 (14), 4558–4564.

Fioramonti, S.A., Martinez, M.J., Pilosof, A.M., Rubiolo, A.C., Santiago, L.G., 2015. Multilayer emulsions as a strategy for linseed oil microencapsulation: effect of pH and alginate concentration. Food Hydrocolloids 43, 8–17.

Frankel, E.N., Satué-Gracia, T., Meyer, A.S., German, J.B., 2002. Oxidative stability of fish and algae oils containing long-chain polyunsaturated fatty acids in bulk and in oil-in-water emulsions. J. Agric. Food Chem. 50 (7), 2094–2099.

Gundstone, F.D., Padley, F.B., 1997. Lipid technologies and applications. CRC Press, USA, New York (Marcel Dekker Inc).

Hogan, S.A., McNamee, B.F., ÓRiordan, E.D., ÓSullivan, M., 2001. Microencapsulating properties of sodium caseinate. J. Agric. Food Chem. 49, 1934–1938.

Horn, A.F., Nielsen, N.S., Andersen, U., Søgaard, L.H., Horsewell, A., Jacobsen, C., 2011. Oxidative stability of 70% fish oil-in-water emulsions: impact of emulsifiers and pH. Eur. J. Lipid Sci. Technol. 113 (10), 1243–1257.

Horn, A.F., Nielsen, N.S., Jensen, L.S., Horsewell, A., Jacobsen, C., 2012. The choice of homogenisation equipment affects lipid oxidation in emulsions. Food Chem. 134 (2), 803–810.

Hu, M., McClements, D.J., Decker, E.A., 2003. Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein isolate. J. Agric. Food Chem. 51 (6), 1696–1700.

Huck-Iriart, C., Álvarez-Cerimedo, M.S., Candal, R.J., Herrera, M.L., 2011. Structures and stability of lipid emulsions formulated with sodium caseinate. Curr. Opin. Colloid Interface Sci. 16 (5), 412–420.

IUPAC, 1992. Standard methods for the analysis of oils, fats and derivates. In: Paquot, C., Hautfenne, A. (Eds.), International Union of Pure and Applied Chemistry, Oxford, seventh ed. Blackwell Scientific Publications Inc., England, Oxford.

Ixtaina, V.Y., Martínez, M.L., Spotorno, V., Mateo, C.M., Maestri, D.M., Diehl, B.W., et al., 2011. Characterization of chia seed oils obtained by pressing and solvent extraction. J. Food Compos. Anal. 24 (2), 166–174.

Ixtaina, V.Y., Nolasco, S.M., Tomás, M.C., 2012. Oxidative stability of chia (*Salvia hispanica* L.) seed oil: effect of antioxidants and storage conditions. J. Am. Oil. Chem. Soc. 89, 1077–1090.

Ixtaina, V.Y., Julio, L.M., Wagner, J.R., Nolasco, S.M., Tomas, M.C., 2015. Physicochemical characterization and stability of chia oil microencapsulated with sodium caseinate and lactose by spray drying. Powder Technol. 271, 26–34.

Komprda, T., Zorníková, G., Rozíková, V., Borkovcová, M., Przywarová, A., 2013. The effect of dietary *Salvia hispanica* seed on the content of n-3 long-chain polyunsaturated fatty acids in tissues of selected animal species, including edible insects. J. Food Compos. Anal. 32 (1), 36–43.

Kuhn, K.R., Cunha, R.L., 2012. Flaxseed oil – whey protein isolate emulsions: effect of high pressure homogenization. J. Food Eng. 111, 449–457.

Let, M.B., Jacobsen, C., Sørensen, A.D.M., Meyer, A.S., 2007. Homogenization conditions affect the oxidative stability of fish oil enriched milk emulsions: lipid oxidation. J. Agric. Food Chem. 55 (5), 1773–1780.

Lethuaut, L., Métro, F., Genot, C., 2002. Effect of droplet size on lipid oxidation rates of oil-in-water emulsions stabilized by protein. J. Am. Oil Chem. Soc. 79 (5), 425–430.

Luna Pizarro, P., Almeida, E.L., Sammán, N.C., Chang, Y.K., 2013. Evaluation of whole chia (*Salvia hispanica* L.) flour and hydrogenated vegetable fat in pound cake. LWT – Food Sci. Technol. 54 (1), 73–79.

Marineli, R.d.S., Moraes, É.A., Lenquiste, S.A., Godoy, A.T., Eberlin, M.N., Maróstica Jr., M.R., 2014. Chemical characterization and antioxidant potential of Chilean chia seeds and oil (*Salvia hispanica* L.). LWT – Food Sci. Technol. 59 (2), 1304–1310.

Martínez-Cruz, O., Paredes-López, O., 2014. Phytochemical profile and nutraceutical potential of chia seeds (*Salvia hispanica* L.) by ultra high performance liquid chromatography. J. Chromatogr. A 1346, 43–48.

McClements, D.J., 2004. Protein-stabilized emulsions. Curr. Opin. Colloid Interface Sci. 9 (5), 305–313.

McClements, D.J., 2005. Food Emulsions: Principles, Practice, and Techniques. CRC Press.

McClements, D.J., 2010. Emulsion design to improve the delivery of functional lipophilic components. Annu. Rev. Food Sci. Technol. 1, 241–269.

McClements, D.J., Weiss, J., (2005). Lipid emulsions. Bailey's Industrial Oil and Fat Products.

Nakaya, K., Ushio, H., Matsukawa, S., Shimizu, M., Ohshima, T., 2005. Effects of droplet size on the oxidative stability of oil-in-water emulsions. Lipids 40 (5), 501–507.

O' Dwyer, S.P., O' Beirne, D., Eidhin, D.N., O' Kennedy, B.T., 2013. Effects of sodium caseinate concentration and storage conditions on the oxidative stability of oil-in-water emulsions. Food Chem. 138 (2–3), 1145–1152.

Pan, L., Tomás, M., Añón, M., 2002. Effect of sunflower lecithins on the stability of water-in-oil and oil-in-water emulsions. J. Surfactants Deterg. 5 (2), 135–143.

Qian, C., McClements, D.J., 2011. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: factors affecting particle size. Food Hydrocolloids 25 (5), 1000–1008.

Vega, C., Douglas Goff, H., Roos, Y.H., 2007. Casein molecular assembly affects the properties of milk fat emulsions encapsulated in lactose or trehalose matrices. Int. Dairy J. 17 (6), 683–695.

Velasco, J., Marmesat, S., Dobarganes, C., Márquez-Ruiz, G., 2006. Heterogeneous aspects of lipid oxidation in dried microencapsulated oils. J. Agric. Food Chem. 54 (5), 1722–1729.