

Development and characterization of functional O/W emulsions with chia seed (*Salvia hispanica* L.) by-products

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Abstract The present work investigated the physicochemical properties of O/W emulsions containing functional ingredients (oil with high ω -3 fatty acid content, protein and/or soluble fiber) from chia seeds. The effect of different protein-carbohydrate combinations (sodium caseinate and lactose, sodium caseinate and maltodextrin, chia protein-rich fraction and maltodextrin) and the presence of chia mucilage (0 and 0.2 % wt/wt) in the aqueous phase of chia O/W emulsions was studied as a function of droplet size distribution, Sauter mean diameter, ζ -potential, rheological properties and backscattering profiles. The use of sodium caseinate in combination with lactose or maltodextrin produced chia O/W emulsions with small droplet size (0.22–0.27 μ m), high degree of uniformity in droplet size distribution, negatively charged droplets (at pH 6.5), pseudoplastic behavior and high physical stability. Emulsions with chia protein-rich fraction presented wider droplet size distribution and higher D[3,2] values than the previous ones, recording a Newtonian behavior. The addition of chia mucilage affected the physicochemical

properties studied, mainly the rheological characteristics of emulsions.

Keywords Chia seed oil · Chia mucilage · Chia protein-rich fraction · O/W emulsions · Physicochemical properties

Introduction

In the last years, the demand for functional foods with multiple health benefits has increased due to the new trend towards a healthy lifestyle. Functional foods are designed to meet the provision of the basic nutrients as well as to offer the potential of enhanced health or reduce risk of diseases. Advances in food technology resulting in new components, products, processes and packaging have provided the food industry more opportunities for value-added products.

Chia seed (*Salvia hispanica* L.) was one of the staple foods for the civilizations of Central America and Mexico in pre-Columbian times. After many years, this seed has regained its popularity and it became a good ingredient for health food market mainly because it is the highest vegetable source of omega-3 (ω -3) fatty acids known up today. Although chia is not a well-known food, actually its cultivation is not only limited to the American continent, but it is also extended to other areas such Australia and Southeast Asia (Mohd Ali et al. 2012).

Chia oil has ~60 % of α -linolenic acid (ALA), which has been linked to many health benefits, including a low risk of heart disease, cancer, as well as improved brain functions (O'Dwyer et al. 2013). Scientific literature has been reported researches related to the chia oil extraction (Ixtaina et al. 2011a, b, 2015; Martínez et al. 2012), its

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oxidative stability (Ixtaina et al. 2012) and its potential use as vehicle to omega-3 FFA into foods (Ixtaina et al. 2015; Julio et al. 2015; Rodea-González et al. 2012). Furthermore, chia seed possess high levels of dietary fiber (>30 % of total weight) and a protein content in the range of 19–23 % (Sandoval-Oliveros and Paredes-López 2012).

The protein content of chia is higher in comparison to wheat, corn, rice, oat, barley and amaranth grains and its amino acid profile do not have limiting factors in the adult diet (Ayerza and Coates 2005, 2011; Bushway et al. 1981). The isolation and characterization of the chia seed protein fraction has been studied by some authors (Olivos-Lugo et al. 2010; Vázquez-Ovando et al. 2012), while functional properties of chia isolates are just beginning to be investigated. Sandoval-Oliveros and Paredes-López (2012) and Olivos-Lugo et al. (2010) reported that chia protein-rich fraction is constituted by globulins (64.9 %), glutelins (20.2 %), albumins (10.9 %) and prolamines (4.0 %) with a high percentage of glutamic acid, arginine and aspartic acid in chia protein isolates. These aminoacids are important for the immunologic system and the prevention against heart diseases. It has been affirmed that the protein isolate showed a good water-holding capacity (WHC) (4.06 wt/wt) and oil-holding capacity (OHC) (4.04 wt/wt), which could suggest a high proportion of hydrophobic residues. Considering these properties, chia seed proteins could be incorporated as functional ingredient in many processed food products (Olivos-Lugo et al. 2010). Besides, chia seed contains about 5–6 % of mucilage, a complex polysaccharide of high molecular weight composed mainly by D-xylose, D-mannose, D-arabinose, D-glucose, galacturonic and glucuronic acid, stable at temperatures up to 244 °C (Lin et al. 1994; Reyes-Caudillo et al. 2008; Timilsena et al. 2016). The mucilage exudes when the chia seed comes into contact with water and it is able to generate high-viscosity solutions at low concentrations being used as a source of soluble dietary fiber. In this sense, numerous health benefits have been associated with an increased intake of dietary fiber, including reduced risk of coronary heart disease, diabetes, obesity, and different types of cancer (Mann and Cummings 2009). In this context, chia mucilage could be used as a new thickening agent in cosmetics and food industries as well as contributing to the consumer health as a source of soluble dietary fiber (Capitani et al. 2013; Timilsena et al. 2016). However, little information has been reported on the functionality of chia seed mucilage as a stabilizing or thickening agent in food products.

The O/W emulsions are gaining attention in food industry as novel delivery systems for bioactive lipophilic materials and also for development of powder products (microcapsules) from their drying (Hogan et al. 2001). These systems consist of oil droplets in an aqueous phase medium in which proteins, the main emulsifiers, adsorb to

the freshly formed interface of oil droplets created during homogenization. Proteins and polysaccharides are commonly used together in many food products. Sodium caseinate is an important emulsifier because of its ability for rapidly conferring a low interfacial tension during emulsification (Dickinson 1999). In food emulsions, polysaccharides are usually added to increase the viscosity or to obtain a gel-like product retarding the destabilization processes, as well as wall materials in microcapsules obtained by emulsion drying. Lactose is a disaccharide extensively applied in the food industry. Julio et al. (2015) reported that the addition of lactose affected the viscosity, the droplet size, the ζ -potential and the stability of chia O/W emulsions with sodium caseinate. By other hand, polysaccharides such as maltodextrin are frequently used in emulsions to inhibit creaming and/or phase separation by increasing the low shear viscosity (Liang et al. 2014).

In this context, the aim of the present work was to study the effect of different protein–carbohydrate combinations and the presence of chia mucilage on the physicochemical properties of functional O/W emulsions containing chia seed oil.

Materials and methods

Materials

Commercial chia oil was provided by Nutracéutica Sturla S.R.L. (Argentina) (C_{16:0} 9.27 %; C_{18:0} 3.41 %; C_{18:1} 9.37 %; C_{18:2} 17.58 %; C_{18:3} 59.02 %; C_{20:0} 1.36 %) and stored until use at 4 ± 1 °C in amber glass bottles without head space. Casein sodium salt from bovine milk was purchased from Sigma Chemical Company (St. Louis, MO). Maltodextrin DE 13–17 % used was obtained from Productos de Maíz S.A. (Argentina) and D-lactose monohydrate from Anedra (Argentina). All reagents used in this research work were of analytical grade.

Chia protein-rich fraction was obtained by a dry processing of defatted chia flour according to Vázquez-Ovando et al. (2012). Briefly, 500 g of dried flour with a particle size <0.5 mm, was obtained by sieving using a 100 Tyler mesh (140 µm screen) in a stirrer Ro-Tap[®] agitation system for 20 min. The fraction passing through the sieve (sieving) was considered as high protein. The protein, fat, moisture, fiber, ash and nitrogen-free extract (by difference) contents of the protein-rich fraction were 43.0, 0.7, 8.4, 14.1, 8.4 and 25.4 % respectively.

Chia mucilage was obtained from whole chia seeds by modified Segura-Campos et al. (2014) method. Chia seeds were soaked in water at a 1: 20 (wt/v) ratio at 50 °C under constant stirring for 30 min in order to induce the mucilage exudation. The crude mixture containing water, gum and

seeds was frozen at $-20\text{ }^{\circ}\text{C}$ for 12 h and then freeze-drying ($-40\text{ }^{\circ}\text{C}$, 0.133 bar, 72 h) (Labconco, Freezone 18, USA). The material dried was sprayed and separated on a sieve using No. 20 ASTM (0.849 mm) and No. 25 ASTM (0.710 mm) meshes. The proximal composition resulted 10.7, 8.9, 9.1, 3.9, 13.6 and 53.8 % of moisture, ash, protein, fat, fiber and nitrogen-free extract, respectively.

Preparation of emulsions

Oil-in-water emulsions were prepared homogenizing 10 % (wt/wt) of chia oil and 90 % (wt/wt) of aqueous phase solution at room temperature. Six emulsions (30 % total solids) with different aqueous phase compositions were prepared: CL (10 % wt/wt sodium caseinate and 10 % wt/wt lactose), CM (10 % wt/wt sodium caseinate and 10 % wt/wt maltodextrine), PM (10 % wt/wt chia protein-rich fraction and 10 % wt/wt maltodextrine), CL + Mg (10 % wt/wt sodium caseinate, 9.8 % wt/wt lactose and 0.2 wt/wt chia mucilage), CM + Mg (10 % wt/wt sodium caseinate, 9.8 % wt/wt maltodextrine and 0.2 wt/wt chia mucilage) and PM + Mg (10 % wt/wt chia protein-rich fraction, 9.8 % wt/wt maltodextrine and 0.2 wt/wt chia mucilage); the aqueous phase solutions were stored overnight at $4 \pm 1\text{ }^{\circ}\text{C}$ prior to emulsification. All emulsions were produced in two stages. A primary homogenization step was conducted in order to make pre-emulsions with a rotor-stator system Ultraturrax T-25 (Janke and Kunkel GmbH, Staufen, Germany) at 9500 rpm, 1 min. Then, in a second homogenization step, the coarse emulsions were emulsified using a valve high-pressure homogenizer (Panda 2 K, GEA NiroSoavi, Parma, Italy) at 600 bar, 4 passes. The pH of the final emulsions was 6.0–6.5. Nisine 0.0012 % (wt/wt) and potassium sorbate 0.1 % (wt/wt), both food grade additives, were added to the emulsions in order to prevent microbial growth. Emulsions obtained were stored at $4 \pm 1\text{ }^{\circ}\text{C}$ and protected from light for 15 days.

Characterization of emulsions

Droplet size

The droplet size distribution was measured by static light scattering using a laser diffraction Malvern Mastersizer 2000E particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, UK) in a range of 0.1–1000 μm as described previously (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 $\sim 12\%$. The refractive indices 1.47 and 1.33 corresponding to the dispersed and the continuous phase were used. Results

were reported as droplet size distribution and De Sauter ($D_{[3,2]}$) mean diameter, which is very sensitive to the presence of the small droplets, as they have greater specific surface area (Jafari et al. 2013).

$$D_{[3,2]} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (1)$$

where n_i is the number of droplets of diameter d_i .

Additionally, to determine the distribution width of droplet sizes, the dispersion index (Span) was calculated from the following equation:

$$Span = \frac{(D_{0.9} - D_{0.1})}{D_{0.5}} \quad (2)$$

where 90, 10 and 50 % of the oil volume in the emulsions is contained in droplets with diameters below or equal to $D_{0.9}$, $D_{0.1}$ and $D_{0.5}$ respectively.

The droplet size was measured immediately after preparation of emulsions and after 15 days of refrigerated storage.

pH and ζ -potential

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

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 according to Julio et al. (2015).

Rheological measurements

Rheological measurements were carried out with a Haake RS600 controlled stress oscillatory rheometer (Haake, Germany) using a coarse plate–plate sensor system with 1.0 mm gap between plates. Measurements were performed in triplicate at constant temperature ($25 \pm 1\text{ }^{\circ}\text{C}$). The samples were subjected to a logarithmic increasing shear rate with a continuous ramp from 1 to 500 s^{-1} in 2 min, followed by a steady shear at 500 s^{-1} for 1 min, and finally a decreasing shear rate from 500 to 1 s^{-1} in 2 min (Capitani et al. 2015). Flow behavior of emulsions was described by fitting the experimentally measured data to the power law model:

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

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

Emulsion stability

Global stability of emulsions was determined by measurements of dispersed light with a Vertical Scan Analyzer (Quick Scan) (Coulter Corp., Miami, FL) according to Pan et al. (2002). The emulsions were transferred to cylindrical glass tubes and periodically measured during 15 days. 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 $40 \mu\text{m}$.

Experimental design and statistical analysis

A 3×2 fully factorial design, replicated twice, was used to study the effect of different protein–carbohydrate combinations (sodium caseinate and lactose, sodium caseinate and maltodextrin, chia protein-rich fraction and maltodextrin) and the presence of chia mucilage (0 and 0.2 % wt/wt) in the aqueous phase of chia O/W emulsions on each variable studied (droplet size distribution, mean diameter, ζ -potential, rheological properties, backscattering profiles). 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 also analyzed by a unifactorial ANOVA test (95 % level of confidence).

Results and discussion

Droplet size

The droplet size distribution (DSD) curves of chia O/W emulsions are shown in Fig. 1. It can be seen that emulsions stabilized with sodium caseinate presented narrow DSD curves with a mono (CL) or bimodal (CM) shape. However, emulsions prepared using chia protein-rich fraction recorded wider and trimodal DSD with a shift towards larger droplet sizes. In addition, DSD curves presented similar shape but shifted to lower particle sizes when chia mucilage was added, especially noticeable for systems with protein-rich fraction.

The results of Sauter diameter ($D[3,2]$) show that the protein–carbohydrate combination had a very significant

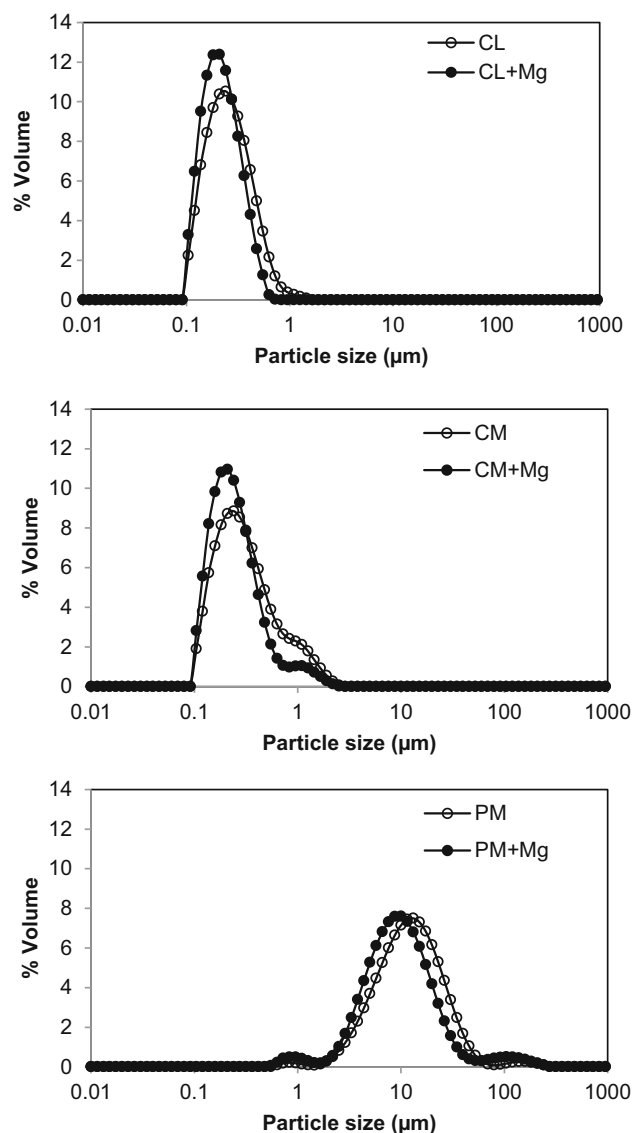


Fig. 1 Effect of different protein–carbohydrate combination and the chia mucilage addition on the particle size distribution (PSD) of chia O/W emulsions ($t = 0$ day). Average values are shown ($n = 2$)

effect ($p \leq 0.01$) on the droplet size of chia O/W emulsions whereas the chia mucilage addition also affected this parameter but in lesser extent ($p \leq 0.05$) (Table 1). The interaction of both factors was also significant ($p \leq 0.05$). The $D[3,2]$ and the polydispersity index (Span) corresponding to initial time and 15 days are summarized in Table 2. As can be seen, systems with sodium caseinate recorded significant ($p \leq 0.05$) smaller droplet sizes and a lower degree of polydispersity (Span) than those obtained with chia protein-rich fraction. At $t = 0$, the $D[3,2]$ values of emulsions with sodium caseinate ranged from 0.22 to $0.27 \mu\text{m}$ and the Span values from 1.07 to 1.46 , denoting the high degree of uniformity associated with these systems. On the other hand, emulsions containing chia

Table 1 Multifactorial analysis of variance (ANOVA) of fully factorial design (3 × 2) of physicochemical properties of chia O/W emulsions

Factor	Degrees of freedom	Sum of squares					
		D _[3,2]	ζ-Potential	n	K	η ₁₀₀	BS _{av0}
Protein–carbohydrate combination (A)	2	92.964**	129.536**	0.1229**	4.8320**	0.072**	350.069**
Chia mucilage addition (B)	1	1.748*	19.152*	0.0439**	4.9227**	0.0722**	10.566*
A × B	2	1.60*	0.467	0.0123**	2.8322**	0.0165**	4.0861
Pure error	6	0.165	1.715	0.0003	0.0145	0.0001	5.721
Total	11						

D_[3,2] mean oil droplet diameters (μm); ζ-potential (mV), *n* flow behavior index; *K* consistency coefficient (Pa sⁿ); η₁₀₀ apparent viscosity at 100 s⁻¹; BS_{av0} initial average backscattering value (%)

* *p* ≤ 0.05; ** *p* ≤ 0.01

Table 2 Volume–surface mean diameter D_[3,2] and polydispersity index (Span) of chia O/W emulsions at different storage times at 4 ± 1 °C

Systems	D _[3,2] (μm)		Span	
	0 day	15 days	0 day	15 days
CL	0.25 ^{aA}	0.27 ^{aA}	1.32 ^{xx}	1.43 ^{xyX}
CM	0.27 ^{aA}	0.26 ^{aA}	1.46 ^{xx}	1.43 ^{xyX}
PM	9.86 ^{cB}	6.99 ^{cA}	2.23 ^{yX}	1.72 ^{yX}
CL + Mg	0.22 ^{aA}	0.22 ^{aA}	1.07 ^{xx}	1.11 ^{xx}
CM + Mg	0.23 ^{aA}	0.24 ^{aA}	1.2 ^{xx}	1.24 ^{xx}
PM + Mg	7.44 ^{bB}	5.99 ^{bA}	2.65 ^{yX}	2.08 ^{zX}
SD	0.01–0.70	0.01–0.13	0.01–0.13	0.01–0.12

SD standard deviation

Different letters indicate significant differences (*p* ≤ 0.05). Small letters: differences in the same column. Capital letters: differences in the same line for the different storage times

protein-rich fraction presented droplet sizes between 7.44 and 9.86 μm and Span values from 2.23 to 2.65 due to presence of bigger particle populations. The type and concentration of emulsifier agent prior to homogenization has an important influence on the size of the droplets produced (Qian and McClements 2011). In this case, the considerably larger droplet size achieved in emulsions with chia protein-rich fraction could be due to its lower available protein level at the chemical environment conditions which would not be enough to cover the surface of smaller oil droplets formed. Some researchers indicated that when protein content is limited there is no longer sufficient protein to fully stabilize the droplet interface, and therefore larger particles may be formed during homogenization (Day et al. 2007; Dickinson 2003). Considering the emulsifying properties, it is well known that sodium caseinate is a flexible protein more effective to reduce the interfacial tension and unfolds at the interface more rapidly than globular proteins (Dickinson and McClements 1995). Thus,

it is expected that sodium caseinate plays a major role as an emulsifier because it presents a higher hydrophobicity, lower surface tension and higher structural flexibility than chia proteins which are mainly constituted by globular proteins (Qian and McClements 2011; Sandoval-Oliveros and Paredes-López 2012).

In addition, smaller (*p* ≤ 0.05) droplet sizes for emulsions with PM + Mg than PM systems were obtained (Table 2; Fig. 1). This fact could be related to the contribution of chia mucilage through the increase of viscosity and formation of a network that reduces the movement of oil droplets, their collision and coalescence. This effect was more important for this type of emulsions than sodium caseinate-stabilized systems probably due to the lower content of non-adsorbed chia protein in the continuous phase.

Droplet size of chia O/W emulsions did not show significant variations during the storage period, except for emulsions with chia protein-rich fraction. In a similar way, no significant differences (*p* > 0.05) were observed in Span as a function of storage time.

ζ-Potential

ζ-potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed droplet. The numerical value of ζ-potential can be related to the stability of emulsions. Emulsions with high absolute ζ-potential (higher than +30 mV or lower than -30 mV) are electrostatically stabilized while emulsions with low absolute ζ-potential tend to coagulate or flocculate (Wang et al. 2011).

The droplet charge of chia O/W emulsions at pH 6–6.5 was negative for all systems. This electrical charge has been attributed to the ionized groups from the protein when the pH was above the isoelectric point (pI). Both the protein–carbohydrate combination used and chia mucilage addition had significant (*p* ≤ 0.05) effects on the ζ-

potential, being the former the factor with the major influence (Table 1). In absence of chia mucilage, the electrical charge the droplets of sodium caseinate-stabilized emulsions was approximately -35 and -31 mV for CL and CM, respectively. A significant ($p \leq 0.05$) decrease in the magnitude of the droplet charge with the replacement of lactose by maltodextrin was found. This fact could be attributed to the emulsifier–maltodextrin interactions at the surface of the emulsion droplets. It could be possible that the maltodextrin molecules bound anionic surface active groups that were originally associated with the chia oil droplets, thereby reducing the negative charge on the oil droplets (Klinkesorn et al. 2004). In addition, the droplets coated by chia protein-rich fraction presented a negative charge of approximately -23 mV, probably due to the presence of functional groups negatively charged in the protein structure, mainly glutamic and aspartic acids.

The net charge on the droplets became significantly ($p \leq 0.05$) less negative when chia mucilage was incorporated. This fact could be attributed to possible charge suppression by electrostatic associations between polypeptide chains and certain charged groups of the chia mucilage.

Rheological measurements

The rheological properties of chia O/W emulsions were very significantly ($p \leq 0.01$) dependent on the protein–carbohydrate combination, chia mucilage addition and the interaction between both factors, as can be seen in Table 1.

Experimental data of chia O/W emulsions flow curves were fitted to the power law model and their parameters (n , flow behavior index and K , consistency coefficient) were calculated (Table 3). In all cases, the values of determination coefficient (R^2), used as criteria of the goodness of model fit, were higher than 0.98. Differences in the flow

Table 3 Power law parameters (n , flow behavior index; K , consistency coefficient) and apparent viscosity at 100 s^{-1} (η_{100}) for chia O/W emulsions at $25 \pm 0.3 \text{ }^\circ\text{C}$

Systems	Power law		η_{100} (Pa s)
	K (Pa s ^{n})	n	
CL	0.234 ^{ab}	0.866 ^c	0.124 ^b
CM	0.537 ^{bc}	0.760 ^b	0.173 ^c
PM	0.007 ^a	1.000 ^d	0.007 ^a
CL + Mg	1.007 ^c	0.700 ^b	0.261 ^d
CM + Mg	3.905 ^d	0.535 ^a	0.464 ^e
PM + Mg	0.015 ^a	1.000 ^d	0.015 ^a
SD	0.001–0.025	0.001–0.007	0.003–0.030

SD standard deviation

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

behavior of different chia O/W emulsions could be evidenced. According to Table 3, emulsions prepared with sodium caseinate recorded values of $n < 1$ denoting pseudoplastic or shear-thinning behavior. The pseudoplasticity of sodium caseinate-stabilized systems, was associated with the mechanical disruption of the structure network (aqueous phase trapped within the inter-droplet structure) at large applied stresses. Also, these systems presented different degrees of shear-thinning behavior indicated by the flow behavior index (n), which decreases when pseudoplasticity increases. Thus, emulsions with maltodextrin were found to be more pseudoplastic than those with lactose. On the other hand, emulsions with protein-rich fraction presented a Newtonian behavior ($n = 1$) (Table 3).

Regarding the dependence of the flow behavior of chia O/W emulsions on shearing time, significant differences ($p \leq 0.05$) were found in the parameters between the upward and downward of all systems with the addition of chia mucilage (data not shown).

The apparent viscosities of different systems at 100 s^{-1} of shear rate (η_{100}), considered as a typical value of food processes such as flow through a pipe, stirring or mastication (McClements 2004), are presented in Table 3. It was observed that systems with chia protein-rich fraction recorded very lower values ($p \leq 0.05$) of this parameter compared to sodium caseinate-stabilized emulsions. This fact could be related to the sodium caseinate level which was higher than that required to completely cover the droplet surface; therefore, a large amount of non-adsorbed sodium caseinate remains in the continuous phase of these systems. This situation would produce an enhancement of the continuous phase viscosity and the depletion energy, which could also lead to the formation of aggregates and a transient emulsion network structure (Julio et al. 2015). An increase of η_{100} was observed when maltodextrin was used instead of lactose with sodium caseinate. Besides, a significant ($p \leq 0.05$) enhancement of apparent viscosity of emulsions with sodium caseinate was evidenced when chia mucilage was added, probably related to the composition and functional properties of chia mucilage (Table 3). These results are in agreement with Timilsena et al. (2016) who reported that the high viscosity of chia seed gum solution can be attributed to the presence of 4-*O*-methyl glucuronic acid substituent which forms inter-molecular chain entanglement in aqueous medium.

Emulsion stability

The Table 1 shows that the protein–carbohydrate combination presented a very significant influence ($p \leq 0.01$) followed by the mucilage addition ($p \leq 0.05$) on BS_{av0} . The average values of initial BS along the entire tube

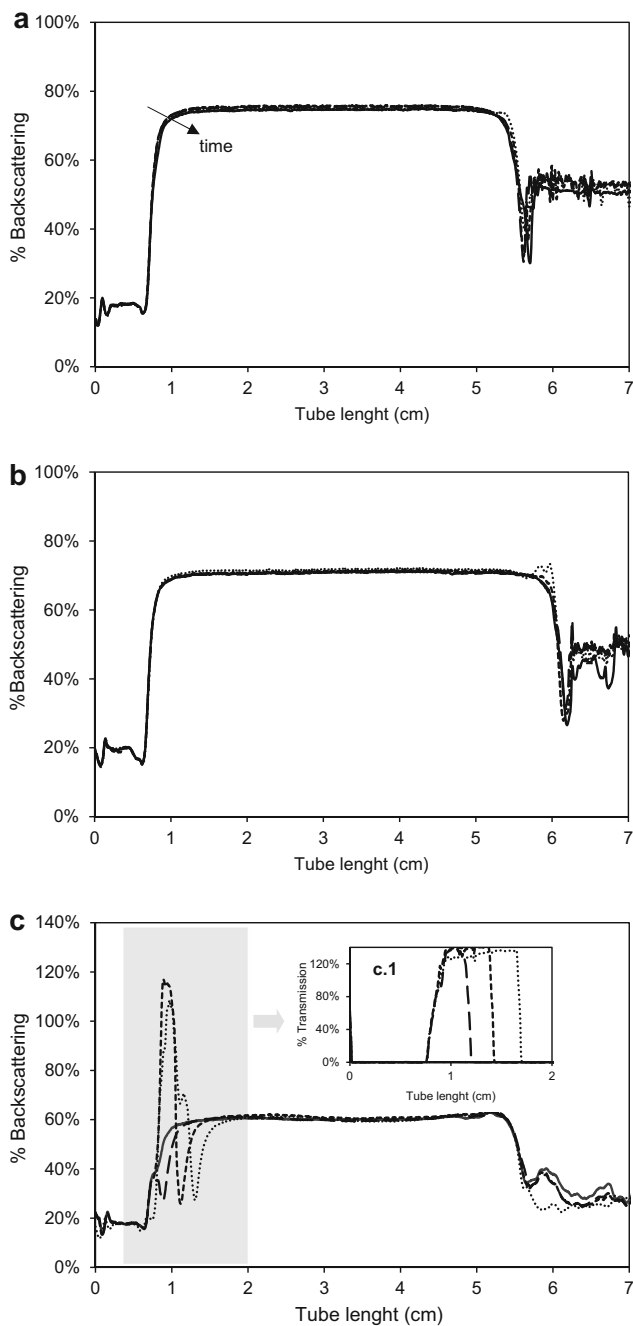


Fig. 2 Backscattering profiles (%BS vs. tube length) of **a** CL, **b** CM and **c** PM chia O/W emulsions and transmission profile corresponding to the bottom of the tube (*shadowed area*) for PM chia O/W emulsions (c.1) as a function of storage: (*straight lines*) initial time, (*dash lines*) 5 days, (*dark dotted lines*) 10 days and (*light dotted lines*) 15 days. Average values ($n = 2$)

(BS_{av0} , from BS profile at $t = 0$) were 63, 59 and 49 % for systems CL, CM and PM, respectively. Taking into account that at $t = 0$ the emulsions distribution of particles is homogeneous, the BS_{av0} is directly dependent on the mean droplet diameter. BS flux increases with the droplet mean diameter when the droplet size is smaller than the

incident wavelength (λ) and it decreases with the mean diameter for particles larger than the incident wavelength (Mengual et al. 1999). Thus, the significant ($p \leq 0.05$) lower BS_{av0} value of protein-rich fraction stabilized emulsions in contrast to sodium caseinate systems could be due to a droplet size higher than λ ($0.8 \mu\text{m}$).

The global stability of chia O/W emulsions was examined during 15 days of storage through the optical characterization method with a Vertical Scan Analyzer (Quick Scan). The evolution of the backscattering (BS) profiles as a function of the sample height (total height = 65 mm) for the different chia O/W emulsions are shown in Fig. 2. The backscattering patterns constitute the macroscopic fingerprint of the emulsion sample at a given time (Mengual et al. 1999) and enabled to examine the migration phenomena of oil droplets. In this sense, emulsions stabilized with sodium caseinate exhibited a high global stability recording no significant changes in BS profiles during the entire period of storage (Fig. 2a, b). This fact could be associated with the high viscosity level and small droplet size of these systems, which drastically reduced the mobility of the chia oil droplets and hence their upward movement according to the Stokes law, supported by the significant correlation found: BS_{av0} versus η_{100} ($r = 0.67$; $p = 0.0157$) and BS_{av0} versus $D[3,2]$ ($r = -0.96$; $p = 0.0000$). On the other hand, clarification was detected in the bottom of the sample tube after 5 days of storage for systems with protein-rich fraction, which could be better visualized through an increase in the transmission ratio at the bottom of the sample tube (Fig. 2c–c.1). This behavior could be associated with the free mobility of the droplets and the high degree of particle interaction, caused by the weak viscous forces in the aqueous phase with chia protein and a low net charge of these emulsion droplets. In this sense, a positive correlation was found between BS_{av0} and ζ -potential ($r = 0.83$; $p = 0.0008$).

Regarding the addition of chia mucilage, emulsions presented similar BS profiles than those without mucilage, recording an increase of $\sim 5\%$ in the BS_{av0} of CM + Mg and PM + Mg systems (data not shown).

Conclusion

O/W emulsions with functional ingredients (oil with high ω -3 fatty acid content, protein fraction and/or mucilage) from chia seeds were developed and characterized investigating the influence of different aqueous phase composition on their physicochemical properties.

High physical stability was achieved for chia O/W emulsions with sodium caseinate-lactose or sodium caseinate-maltodextrin with a small droplet size and a low polydispersity, negatively charged droplets (at pH 6.5), and

pseudoplastic behavior. A decrease in the net charge of ζ -potential and an increase of η_{100} were observed when maltodextrin was used instead of lactose.

In contrast, systems with chia protein-rich fraction presented wider droplet size distributions and higher Sauter mean diameter than emulsions with sodium caseinate, recording a Newtonian behavior. A limited stability was detected after 5 days of storage by a clarification process.

The impact of the addition of chia mucilage—a soluble dietary fiber—was reflected mainly on the rheological characteristics of emulsions. Thus, its use increased the viscosity of the continuous phase slowing down the movement of oil droplets with the consequent improvement on the emulsion stability, evidencing its interesting role as a thickening agent.

This information is particularly useful for the design and production of O/W emulsions as a ω -3 delivery-systems of chia by-products revaluing the application of these novel ingredients in order to be included in the development of diverse functional foods (emulsion-based, powder foods).

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