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Elucidation of lipid structural characteristics of chia oil emulsion gels by Raman spectroscopy and their relationship with technological properties

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ABSTRACT

Emulsion gels (EGs) offer interesting possibilities for structuring edible oils and obtaining ingredients with solid-like mechanical characteristics and depending on the ingredients used with healthier properties. In that context, the aim of this study was to develop EGs with chia oil, alginate (as cold gelling agent) and different plant derivatives (oat bran, soy protein isolate and chia flour) with both health benefits and appropriate properties for use for example as animal fat replacers in reduced-fat meat products. To that end, technological properties (pH, colour, texture, etc.) and lipid structure, determined by Raman spectroscopy, were investigated in various chia oil emulsion gels (EGs) prepared with chia oil, alginate and soy protein isolate (SEG), oat bran (OEG) or chia flour (CEG). No noticeable release of exudates was observed in chia oil EGs after processing. pH, colour and textural behaviour (evaluated as puncture force and gel strength) differed ($p < 0.05$) depending on the composition of the plant ingredients (soy protein, oat bran or chia flour) that contain these EGs. Raman spectroscopic results, particularly significant differences found in $area_{2800\text{-}3000}$ and I_{2854}/I_{2900}, revealed differences depending on EG composition in the lipid structure and interactions in terms of lipid acyl chain mobility (order/disorder). These lipid structural characteristics of chia oil EGs correlated significantly with a specific textural feature. The results suggest that chia oil EGs could be developed for use as healthier lipid ingredients in new food systems such as healthier meat products.

1. Introduction

The stabilization and structuring of edible oils in order to promote solid-lipid functionality for use as an alternative to animal fat in the development of healthy lipid food (meat) products presents a considerable technical challenge; emulsion gels (EGs) are a promising approach in this regard. An EG is defined as an emulsion with a gel-like network structure and solid-like mechanical properties (Dickinson, 2013; Jiménez-Colmenero et al., 2015). EG preparation essentially involves producing a protein-stabilized emulsion using emulsifying agents and incorporating a gelling agent such as a hydrocolloid or other ingredients with gelling capacity to convert the emulsion into an EG, either by aggregation of emulsion droplets or by gelling of the continuous phase (Dickinson, 2013; Jiménez-Colmenero et al., 2015). The structural state of an oil-in-water (O/W) emulsion gel has been described as a composite network based on a combination of aggregated emulsion droplets and cross-linked biopolymer molecules, this real structural state determining its textural properties (Dickinson, 2012, 2013; Jiménez-Colmenero et al., 2015). The use of cold gelling agents like alginate could be a useful option to formulate EGs due to their ability to form a gel structure (Pintado, Herrero, Jiménez-Colmenero, & Ruiz-Capillas, 2016a; Pintado, Ruiz-Capillas, Jiménez-Colmenero, Carmona, & Herrero, 2015; Roopa & Bhattacharya, 2010). Another promising approach to EG formulation is to replace synthetic emulsifiers or stabilizers with natural ones, given that in recent years there has been strong consumer demand for foods with “clean labels” (Baines & Seal, 2012). Vegetable ingredients like soy, oat and their derivatives (isolate, flour, oil, etc.) could be a promising option in this connection (Laine et al., 2011; Nishinari, Fang, Guo, & Phillips, 2014; Olivos-Lugo, Valdivia-Lopez, & Tecante, 2010). One advantage is that these plant derivative ingredients contain various healthy

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bioactive compounds (Arendt & Zannini, 2013; Nishinari et al., 2014; Valdivia-López & Tecante, 2015). In particular chia, an oilseed plant, is a good natural source of omega-3 fatty acids (mainly α-linolenic acid) and insoluble fibre (Mohd Ali et al., 2012; Munoz, Cobos, Diaz, & Aguilera, 2013; Reyes-Caudillo, Tecante, & Valdivia-Lopez, 2008), and oat provides useful soluble dietary fibre, primarily β-glucan which is associated with several health benefits (Arendt & Zannini, 2013; Menon et al., 2016; Sterna, Zute, & Brunava, 2016). Another advantage is their technological properties. The proteins in these plant ingredients generally have high water-holding capacity and appropriate emulsifying activity to provide high emulsion stability; and in certain conditions they can also aid gel formation. Moreover, some of the ingredients they contain, such as fibre in chia and oat (mainly β-glucan), have useful gelling properties (Brummer et al., 2014; Burkus & Temelli, 2000; Capitani, Nolasco, & Tomás, 2016; Coorey, Tjoe, & Jayasena, 2014; Kontogiorgos, Biliaderis, Kiosseoglou, & Doxastakis, 2004; Lazaridou & Biliaderis, 2007; Olivos-Lugo et al., 2010; Santipanichwong & Suphantharika, 2009; Timilsena, Adhikari, Barrow, & Adhikari, 2016). Some EGs have been developed containing derivatives of soy, chia or oat and other plant ingredients (Hou, Guo, Wang, & Yang, 2016; Jiménez-Colmenero, Herrero, Pintado, Solas, & Ruiz-Capillas, 2010; Pintado et al., 2016a; Pintado et al., 2015; Singh et al., 2016; Wang et al., 2017). It has been shown that the different ingredients used in the formation of EGs affect the interactions and structural state and mechanical properties that determine their stability and behaviour (Dickinson, 2012, 2013; Pintado et al., 2015). This is because the properties of EGs are the net result of complex interactions between the various components making them. An understanding of the relationship between technological and structural characteristics of EGs as affected by the ingredients used to prepare them can help us to select the most suitable one so that these EGs can help to improve or maintain the quality of the food to which they are added. Particularly important in this regard is their use as animal fat replacers, for instance in the development of healthier meat products without prejudicing the final properties of the product (Herrero, Carmona, Pintado, Jiménez-Colmenero, & Ruiz-Capillas, 2012; Poyato, Ansorena, Berasategi, Navarro-Blasco, & Astiasaran, 2014; Poyato, Astiasaran, Barriuso, & Ansorena, 2015; Pintado et al., 2016b).

Raman spectroscopy is a direct, non-invasive technique which has proven to be a powerful tool providing structural information on the various components (proteins, lipids, carbohydrates, etc.) (Herrero, 2008a, b; Herrero, Carmona, Jiménez-Colmenero, & Ruiz-Capillas, 2014; Muik, Lendl, Molina-Díaz, & Ayora-Cañada, 2003) involved in the formulation of EGs. In that connection, it is important to note the role of the structure and the interactions of the lipid phase in the stability of EGs and their technological properties (Dickinson, 2013; Pintado et al., 2015).

Based on the above considerations, the objective of this study was to develop oil-in-water (O/W) EGs formulated with chia oil, alginate (as cold gelling agent) and different plant derivatives (oat bran, soy protein isolate and chia flour) with health benefits and appropriate technological properties for use as healthier-lipid ingredients in low-calorie and reduced-fat products (specifically meat products). In these EGs two main aspects were investigated depending on the composition of these different plant ingredients, their technological characteristics (since these affect quality properties of the products in which they will be added), their lipid structural characteristics and the interactions (using Raman spectroscopy). The ultimate objective was to establish possible relationships between technological and lipid structural properties to better understand the stability and behaviour of these chia oil EGs on the basis of the plant ingredients used in their formulation.

2. Materials and methods

2.1. Materials

The ingredients used to make chia oil emulsion gels (EGs) were: oat bran (OatWell® 22%, Zeus Química, S.A. Barcelona, Spain) with 20% protein, 20% carbohydrates, 44% total dietary fibre (of which 22% β-glucan soluble fibre) and 5.1% fat according to the supplier; soy protein isolate (SPI) (90% protein content) (Manuel Riesgo SA, Madrid, Spain); chia flour (*Salvia hispanica* L.) (Primaria Premium Raw Materials, S. L. Valencia, Spain) with 22% protein, 5.1% carbohydrates, 31% fat and 30% dietary fibre according to information provided by the supplier; sodium alginate (90% carbohydrate content) (Tradissimo, TRADES S.A., Barcelona Spain); calcium sulphate (CS) (Panreac Química, S.A. Madrid, Spain), tetra-sodium pyrophosphate anhydrous PRS (SP) (Panreac Química, S.A. Madrid, Spain) and unrefined chia oil (Primaria Premium Raw Materials, S.L; Valencia, Spain) containing a total of approximately 85 g of PUFA/100 g chia oil (57–65 g α-linolenic/100 g chia oil).

2.2. Preparation of chia oil emulsion gels

Three different types of chia oil EGs were prepared with 40% chia oil, a gelling agent based on alginate (1% sodium alginate, 1% $CaSO_4$ and 0.75% sodium pyrophosphate), 34.25% water and 23% of: a) oat bran (OEG), b) SPI (SEG) or c) chia flour (CEG), (Table 1).

These EGs were prepared according to Pintado et al. (2015). All ingredients were added by weight. Briefly, first for each type of EG, oat bran, SPI or chia flour, was mixed in a homogenizer (Thermomix TM 31, VorwerkEspaña M.S.L., S.C, Madrid, Spain) with water (30 s, approx. 5600 rpm), then the alginate-based gelling agent was added and mixed (15 s, approx. 5600 rpm). Chia oil was gradually added to this mixture with the homogenizer in operation (5600 rpm). Finally, each type of EG was placed in a metal container under pressure to compact it and prevent air bubbles, and stored in a chilled room at 2 °C for 20 h. After this time the metal containers were removed and the EGs analysed. Each type of sample was prepared in triplicate.

The same mixtures of oat bran, SPI or chia flour and the gelling agent (alginate) in aqueous solution (without added chia oil) were prepared for use as references for spectroscopic measurements.

Table 1
Formulation (%) of chia oil emulsion gels.

Samples[a]	Chia oil	Gelling agent			Water	Oat bran	SPI	Chia flour
		Sodium alginate	$CaSO_4$	Sodium pyrophosphate				
OEG	40	1	1	0.75	34.25	23		
SEG	40	1	1	0.75	34.25		23	
CEG	40	1	1	0.75	34.25			23

[a] Chia oil emulsion gels prepared with chia oil, a gelling agent based on alginate, water and: 1) oat bran namely OEG; 2) soy protein isolate (SPI) namely SEG; 3) chia flour namely CEG.

These samples were denominated OG, SG and CG, for oat bran, SPI and chia flour respectively.

2.3. Technological properties

2.3.1. Fluid release and pH measurement

Water and fat binding properties were estimated as fluid released (FR), evaluated in samples by weight difference (%) between initial and final sample weight after preparation.

pH was measured in triplicate using a 827 Metrohm pH-meter (MetrohmAG, Switzerland) on homogenates of sample in distilled water in a ratio of 1:10 w/v.

2.3.2. Colour measurement

Colour (CIE-LAB tristimulus values, lightness, L*; redness, a* and yellowness, b*) was evaluated on a Chroma Meter CR-400 (Konica Minolta Business Technologies, Inc., Tokyo, Japan). Determinations were carried out on cross-sections of sample. Ten determinations were performed from each chia oil EG.

2.3.3. Penetration test

Penetration tests were performed at about 22 °C using a TA-XT.plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) with the Texture Exponent program. A load cell of 5 kg was employed. Penetration test was performed according to Herrero, Carmona, Pintado, Jiménez-Colmenero, and Ruiz-Capillas (2011b). Analysis was carried out with a 4 mm diameter cylindrical stainless steel plunger at a velocity of 0.8 mm/s and force exerted at 10 mm. The textural parameters of each sample derived from their force-deformation curves were: (a) penetration force (PF, N) at the point of gel rupture and (b) gel strength (GS, N mm), which is defined as the area enclosed by the force-deformation curve at the point of gel rupture. Six measurements per sample were performed.

2.4. Structural properties

2.4.1. FT-Raman spectroscopic analysis

The following Raman spectra were measured: a) spectra of the oat bran, SPI and chia flour, with the gelling agent in aqueous solution used as reference (OG, SG and CG,); b) spectra of liquid chia oil (CO); and c) spectra of the different types of chia oil EG (OEG, SEG and CEG).

Portions of the different samples were transferred to quartz cuvettes (ST-1/Q/10) (TEKNOKROMA, Barcelona, Spain) to fill them to a length of 1 cm. 1500 scans were recorded for each sample. This procedure was carried out in triplicate, giving a total of 4500 scans per sample. Measurements were performed in triplicate for each sample prepared from each chia oil EG. Spectra were excited with the 1064 nm Nd: YAG laser line and recorded on a Bruker RFS 100/S FT-spectrometer. The scattered radiation was collected at 180° to the source, and frequency-dependent scattering of the Raman spectra, produced by the spectrometer, was corrected by multiplying point by point with $(\text{mlaser/m})^4$. The influence of the optics on the spectrometer was eliminated by using the Raman correction command from the Opus 2.2 software (Bruker, Karlsruhe, Germany). Reported frequencies are accurate to $\pm 0.5\ \text{cm}^{-1}$, as deduced from frequency standards measured in the spectrometer. Raman spectra were resolved at $4\ \text{cm}^{-1}$ resolution with a liquid nitrogen-cooled Ge detector. Samples were illuminated by laser power at 300 mW.

Raman spectra were processed using the Bruker Opus 2.2 and Grams/AI version 9.1 (Thermo Electron Corporation, USA) software.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was performed to evaluate the statistical significance ($p < 0.05$) of the effect of chia oil EG composition using the SPSS Statistics general linear model (GLM) procedure (v.22, IBM SPSS Inc.; Chicago, IL, USA). Least squares differences were used for comparison of mean values among formulations and Tukey's HSD test to identify significant differences ($p < 0.05$) between formulations. Pearson product moment correlations (R) were performed to determine statistically significant relationships between data obtained by pH, colour, penetration test (puncture force and gel strength) and Raman spectroscopy analysis focused on pairs of variables. Only significant relationships are reported. P-values were used to test the statistical significance of the estimated correlations.

3. Results and discussion

3.1. Concerns about chia oil EG composition

In order to understand differences in chia oil EG characteristics, some considerations need to be set out regarding their composition as estimated from formulations (Table 1).

Protein content of the samples (Table 1) was about 4.6, 5.1, and 20.7% for OEG, CEG and SEG respectively. Soy protein is composed mainly of two globular protein factions, designated as 11S (glycinin) and 7S (β- and γ-conglycinin), which constitute about 40% and 28% respectively of the total proteins (Fukushima, 1991). Oat bran proteins consist mainly of globulins, although they also contain smaller proportions of albumins, glutelins and prolamines (Klose & Arendt, 2012). The main protein fraction of chia is also composed of globulins (about 52%) containing mostly 11S and 7S proteins as major and minor components (Sandoval-Oliveros & Paredes-Lopez, 2013). SPI, oat and chia proteins are well balanced in amino acid composition (Ayerza & Coates, 2011; Fukushima, 1991; Klose & Arendt, 2012); they generally possess useful technological properties, including high emulsifying stability (Laine et al., 2011; Nishinari et al., 2014; Olivos-Lugo et al., 2010).

The fat content of chia oil EGs was compressed to about 40–47% according to formulation (Table 1). The fat in these samples derived mainly from chia oil (40%), although in EGs containing oat (OEG) or chia (CEG), the latter compounds made up approximately 1 or 7% of the fat respectively. Chia oil and flour are a plant source of omega-3 fatty acids (mainly α-linolenic acid); they contain relatively low levels of saturated fatty acids and confer certain health benefits (Ayerza & Coates, 2005).

Both chia oil EGs, prepared with oat bran or chia flour, also contained carbohydrates (about 4.6 and 1.1%) and dietary fibre (about 10.1 and 6.9%). However, chia flour contains mainly insoluble fibre while oat bran contains a considerable amount of soluble fibre (β-glucan) (Arendt & Zannini, 2013; Reyes-Caudillo et al., 2008; Vázquez-Ovando, Rosado-Rubio, Chel-Guerrero, & Betancur-Ancona, 2009). Oat β-glucan in particular is associated with several health benefits (Wolever et al., 2010) and offers promising possibilities as a gelling agent depending on the conditions of use (Brummer et al., 2014; Burkus & Temelli, 2000; Kontogiorgos et al., 2004; Lazaridou & Biliaderis, 2007; Santipanichwong & Suphantharika, 2009).

3.2. Technological properties

3.2.1. Fluid release and pH measurement

Binding (water and fat) properties of the different chia oil EGs were optimal, with no noticeable (<0.1%) total fluid release (TFR) of exudates after processing. Similar results have been reported in

olive oil EGs (Herrero, Carmona, Pintado, Jiménez-Colmenero, & Ruiz-Capillas, 2011a; Herrero et al., 2011b). It has been observed that oil-in-water EGs formed with a combination of different proteins (caseinate, SPI, etc.) and a cold gelling agent (MTG) showed a compact network structure with numerous small cavities associated with good water and fat binding properties (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, 2010).

pH values of chia oil EGs could have an impact on final food products where they are incorporated, for instance as animal fat replacers in meat products. Table 2 shows that pH values of the different samples were affected ($p < 0.05$) by formulation. The highest ($p < 0.05$) pH values (7.29) were observed in chia oil EGs containing SPI (SEG), (Table 2). All pH values were within the value ranges reported for other EGs prepared with this protein source (Jiménez-Colmenero et al., 2010; Pintado et al., 2016a) and with pork backfat (Jiménez-Colmenero et al., 2012), which implies that any of these samples may present limitations for use as animal fat replacers.

3.2.2. Colour

Colour is one of the main factors determining the consumer's choice of foods, including meat products. Therefore, essential to evaluate colour in the development of fat analogues like the chia oil EGs studied in the present work. Table 2 shows colour parameters, which depend significantly on EG formulation. The lightest colour ($p < 0.05$) ($L^* = 75.50$) occurred in EGs containing SPI and the darkest ($p < 0.05$) ($L^* = 65.80$) in samples with oat (OEG) (Table 2). SEG samples registered the lowest ($p < 0.05$) redness ($a^* = 1.27$) and OEG and CEG the highest ($p > 0.05$) ($a^* = 2.33$ and 2.31 respectively). The lowest yellowness ($b^* = 10.50$) was registered in samples containing chia flour (CEG) (Table 2). Since all samples contained the same amount of chia and gelling agent (alginate), the observed differences in colour parameters may be attributed to the other plant ingredients (SPI, oat bran or chia flour) they contained. As in the case of the chia oil EGs considered in this paper reduced brightness and increased redness have been reported in certain bakery products with added oat bran and attributed to oat given that it confers a darker colour and greater redness (Lazaridou & Biliaderis, 2007; Lazaridou, Biliaderis, & Izydorczyk, 2003). Similarly, the colour parameters observed in CEG samples could be influenced by the characteristic darkness of the chia flour present in these EGs (Coelho & Salas-Mellado, 2015; Pintado et al., 2015).

3.2.3. Textural properties: penetration test

Texture is another crucial aspect of the technological aptitude of these materials for use as fat analogues and for consumer acceptance or rejection of products containing them. With this in mind, textural properties were evaluated in terms of penetration force (PF) and gel strength (GS) of the chia oil EGs formulated (Table 2). In all samples force-deformation curves showed a breaking point characteristic of a gel structure, but their textural behaviour differed significantly depending on their formulation (Table 1). PF and GS were highest ($p < 0.05$) ($PF = 3.21$ and $GS = 28.70$) in the chia oil EGs containing oat (OEG) and lowest ($p < 0.05$) ($PF = 1.21$ and $GS = 11.90$) in CEG samples (Table 2). It has been shown that the rheological behaviour of emulsions differs widely depending on their composition, structure, droplet interactions, droplet size, etc. (Dickinson, 2012; McClements, Decker, & Weiss, 2007). As formulated, the three EGs studied contained the same proportions of chia oil, gelling agent (alginate) and plant derivatives (oat bran, SPI or chia flour) (Table 1). However, the composition of these ingredients differed in terms of protein content and the presence of other compounds, particularly in the case of oat bran and chia flour. As reported elsewhere, the proteins in the different plant ingredients used possess suitable emulsifying and gel-forming capabilities (Burkus & Temelli, 2000; Nishinari et al., 2014; Olivos-Lugo et al., 2010). However, the formation of a stronger OEG network structure, as evidenced by the highest PF and GS (Table 2), could be related to certain oat compounds such as β-glucan, given the potential effect of oat β-glucan as a stabilizer in emulsion-type food products and its good gelling properties (Burkus & Temelli, 2000). In that respect, a previous study on oat emulsion gels reported an increase in sample firmness with increased oat bran content, attributed mainly to the action of proteins and β-glucan present in oat (Pintado et al., 2016a). Additionally, some authors have shown that β-glucan contributes to the formation of a stronger gelled network (Burkus & Temelli, 2000; Kalinga & Mishra, 2009; Lazaridou & Biliaderis, 2007; Vaikousi, Biliaderis, & Izydorczyk, 2004).

Oil droplet size also has a considerable effect on the rheological properties of emulsion gels (Matsumura, Kang, Sakamoto, Motoki, & Mori, 1993; McClements, Monahan, & Kinsella, 1993). Particularly in whey protein isolate (WPI) gels containing emulsion droplets, it was observed that gel strength decreased as the droplet size increased (McClements et al., 1993). Additionally, in emulsion gels stabilized with chia flour it has been shown that there was a significant negative correlation between emulsion droplet size and PF and GS (Pintado et al., 2015). Taking the above observations into account, it seems that the EG containing oat, which showed the highest PF and GS values (Table 2), could be expected to have the lowest droplet size.

EGs with similar technological properties and composition have been used as animal fat replacers in the development of various healthier meat products (Herrero et al., 2012; Poyato et al., 2014, 2015; Pintado et al., 2016b; Pintado, Herrero, Jiménez-Colmenero, Pasqualin Cavalheiro, & Ruiz-Capillas, 2018).

Table 2
pH values, colour [(L^*) lightness, (a^*) redness and (b^*) yellowness)] and texture parameters [puncture force (PN, N) and gel strength (GS, N mm)] of chia oil emulsion gels.

Parameters	Samples[a]		
	OEG	SEG	CEG
pH	6.93 ± 0.02^{c}	7.29 ± 0.02^{a}	6.99 ± 0.02^{b}
L^*	65.80 ± 0.44^{c}	75.50 ± 0.55^{a}	69.28 ± 0.90^{b}
a^*	2.33 ± 0.05^{a}	1.27 ± 0.10^{b}	2.31 ± 0.19^{a}
b^*	19.41 ± 0.20^{a}	19.20 ± 0.38^{a}	10.50 ± 0.32^{b}
PF	3.21 ± 0.28^{a}	1.91 ± 0.11^{b}	1.21 ± 0.09^{c}
GS	28.70 ± 0.71^{a}	18.04 ± 0.74^{b}	11.90 ± 0.73^{c}

Means ± standard deviation. Different letters in the same row indicate significant differences ($p < 0.05$).
[a] For sample denominations, see Table 1.

3.3. Structural properties

The structure of an emulsion gel and the interactions between its different components may influence its stability and textural properties among other characteristics (Dickinson, 2012; Dickinson & Yamamoto, 1996; Matsumura et al., 1993; McClements et al., 1993; Pintado et al., 2015). Raman spectra analysis of chia oil EGs can provide insights into the molecular interactions involved in stabilization as reported below.

3.3.1. FT-Raman spectroscopic analysis: lipid structural characteristics

One of the spectroscopic regions of interest in Raman studies of molecular systems containing lipids is that of C-H stretching modes (2800-3000 cm^{-1}), which are analysed as follows. In order to eliminate any spectral influence of water and other components (oat bran, SPI, or chia flour) in chia oil EG spectra, the corresponding

spectra of the reference aqueous solutions of these components (OG, SG or CG,) were subtracted from the chia oil EG spectra (OEG, SEG or CEG). For that purpose the 2125 cm^{-1} band of water (Alix, Pedanou, & Berjot, 1988) was eliminated using a subtraction factor so that the intensity peak was not visible. The subtraction of these plant ingredients from this spectral region (2800-3000 cm^{-1}) was confirmed by the disappearance of the characteristic Phe ν−ring band of protein located near 1003 cm^{-1} (Herrero, 2008a, b). The resulting difference spectrum was compared with the chia oil spectrum by normalizing the intensities according to the = CH bending band located near 1267 cm^{-1} (Herrero et al., 2014; Zou et al., 2009).

Fig. 1 shows the different resulting chia oil EGs (OEG, SEG and CEG) and chia oil (liquid form) spectra (used as reference) in the 2800-3000 cm^{-1} region. The spectra show the CH_2 and CH_3 stretching bands that are characteristic of symmetric and asymmetric C-H stretching vibrations of methylene and methyl groups in aliphatic molecules (Levin & Lewis, 1990; Razumas et al., 1996). Thus, the CH_2 symmetric stretching band appears at 2854 cm^{-1}, the CH_2 asymmetric stretching band at 2900 cm^{-1}, and the CH_3 symmetric stretching motion close to 2934 cm^{-1} (Razumas et al., 1996). Intensity of the CH stretching region is considered a powerful tool for investigating the nature of the intermolecular interactions in hydrocarbons (Herrero, 2008a, b; Larsson & Rand, 1973; LiChan, 1996). In this regard, the Raman intensity of the C-H stretching modes in this lipid Raman spectral region (2800-3000 cm^{-1}) decreased with formation of the EGs (Fig. 1). Reduced intensity in the 2800-3000 cm^{-1} region has been attributed to interaction between oil ester molecules and water and/or carbohydrate molecules through the ester carbonyl groups when polysaccharide gels are formed as oil bulking agents (Herrero et al., 2014). Some authors have also studied the interaction of protein and oil at the protein/oil interface and suggested that reduced intensity in the CH symmetric and asymmetric stretching region involved CH (CH_2 and/or CH_3) groups in protein-lipid interactions at the interface (Meng, Chan, Rousseau, & Li-Chan, 2005). All the foregoing suggests that the intensity decrease observed upon formation of the chia oil EGs (Fig. 1) could be attributed to interactions between chia oil ester molecules and protein, water and/or carbohydrates (in the case of EGs containing oat bran or chia flour). In particular, it should be noted that the decrease of intensity in this Raman region was more pronounced in EGs containing oat (Fig. 1) which could be due to stronger interactions of oil acyl chains in these samples (Herrero et al., 2014). These results were confirmed by quantification of the area in this region (2800-3000 cm^{-1}), since OEG registered the lowest ($p < 0.05$) area values (Table 3).

It is assumed that the ratio of the peak-height intensity of the symmetric methyl and methylene stretching band to that of the asymmetric methylene stretching band, $I\nu_s CH_2/I\nu_{as}CH_2$ (I_{2854}/I_{2900}) and $I\nu_sCH_3/I\nu_{as}CH_2$ (I_{2934}/I_{2900}), provides useful information for gauging of lipid packing effects and directly reflects the state of order or disorder of acyl chains in different lipids phases (Herrero et al., 2014; Levin & Lewis, 1990; Razumas et al., 1996). In particular, $I\nu_s CH_2/I\nu_{as}CH_2$ (I_{2854}/I_{2900}) directly monitors acyl chain disorder/order arising from lateral inter-chain interactions (Herrero et al., 2014; Levin & Lewis, 1990; Razumas et al., 1996). For its part, the $I\nu_sCH_3/I\nu_{as}CH_2$ (I_{2934}/I_{2900}) intensity ratio measures effects originating from changes in intra-chain trans/gauche isomerization superimposed on the chain-chain interactions. Changes in the I_{2934}/I_{2900} intensity ratio were non-significant whereas the differences

Table 3
Area values of νCH bands in the 2800-3000 cm^{-1} region ($area_{2800-3000}$) and relative intensity ratio of $I\nu_sCH_2/I\nu_{as}CH_2$ (I_{2854}/I_{2900}), $I\nu_sCH_3/I\nu_{as}CH_2$ (I_{2934}/I_{2900}) and δC = C (I_{1658}) of Raman spectra from chia oil emulsion gels.

Parameters[a]	Samples[b]			
	Chia oil	OEG	SEG	CEG
$area_{2800-3000}$	158.6 ± 1.4[a]	63.7 ± 1.2[c]	75. 95 ± 1.2[b]	72.9 ± 2.0[b]
I_{2854}/I_{2900}	0.96 ± 0.01[a]	0.74 ± 0.02[c]	0.83 ± 0.02[b]	0.82 ± 0.02[b]
I_{2934}/I_{2900}	1.01 ± 0.01[a]	1.01 ± 0.01[a]	1.00 ± 0.02[a]	1.00 ± 0.01[a]
I_{1658}	2.06 ± 0.03[a]	1.44 ± 0.02[b]	1.46 ± 0.021[b]	1.52 ± 0.02[c]

Means ± standard deviation. Different letters in the same row indicate significant differences ($p < 0.05$).
[a] $Area_{2800-3000}$ and I_{1658} values were obtained from spectra normalized by 1267 cm^{-1}band.
[b] For sampledenominations, see Table 1.

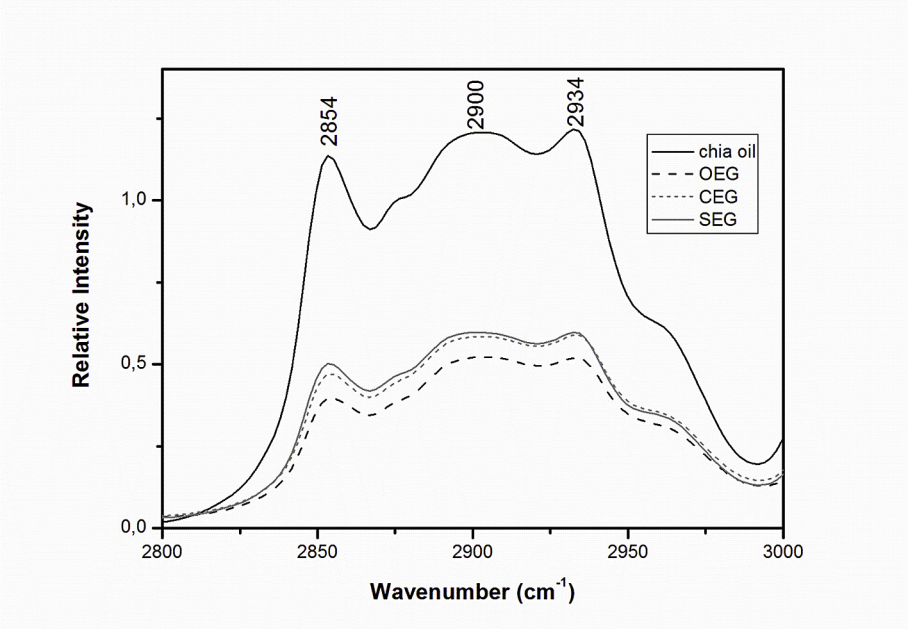

Fig. 1. Raman spectra in the 2800-3000 cm^{-1} region from chia oil (liquid) and chia oil emulsion gels. For sample denomination see Table 1.

observed in the I_{2854}/I_{2900} ratio were significant (Table 3). A lower intensity ratio (I_{2854}/I_{2900}) reflects an increase in the inter-chain vibrational coupling and a decrease in mobility of the acyl chains (Levin & Lewis, 1990; Razumas et al., 1996). Some studies using model multilamellar systems have shown that an I_{2854}/I_{2900} intensity ratio of about 0.77 is characteristic of a bilayer whose chains are hexagonally packed, while a value of 0.98 reflects a normal liquid crystalline phase (Levin & Lewis, 1990). Given this intensity ratio dependence, the results of I_{2854}/I_{2900} (Table 3) indicate significantly higher inter-chain vibrational coupling and lower acyl chain mobility in chia oil EGs than in liquid chia oil. This is to be expected from micelle formations where lipid acyl chains are relatively fixed through hydrogen bonding (Herrero et al., 2014; Razumas et al., 1996). These data were more pronounced in EGs containing oat, where I_{2854}/I_{2900} values were lowest (Table 3). Other authors have reported that this intensity ratio (I_{2854}/I_{2900}) was lower in the case of a milk emulsion than in the separated fat phase and suggested that most of the hydrocarbon chains of the milk fat globule membrane and/or those of the triglyceride molecules adjacent to the membrane are crystalline and close packed, and this packing is of importance for emulsion stability (Larsson, 1976). Additionally, in corn oil emulsions containing lysozyme, lower $I\nu_sCH_2/I\nu_{as}CH_2$ and $I\nu_sCH_3/I\nu_{as}CH_2$ intensity ratios has been related to hydrophobic interactions involving the CH groups of the lipid with protein in the cream layer (Howell, Herman, & Li-Chan, 2001). Some authors have reported the formation of intermolecular hydrogen bonding in soy lecithin emulsion hydrogels (Singh et al., 2016).

These differences in lipid structure and interactions (Table 3) as a function of EG formulation (mainly if they contain oat bran, SPI or chia flour) (Table 1) were accompanied by different textural behaviour (Table 2). In particular, there was a significant negative correlation was found between $area_{2800\text{-}3000}$ ($R = -0.82$; $p < 0.0001$) and I_{2854}/I_{2900} ($R = -0.88$; $p < 0.005$) values and PF. Similarly, a significant negative correlation between $area_{2800\text{-}3000}$ ($R = -0.96$; $p < 0.0001$) and I_{2854}/I_{2900} ($R = -0.90$; $p < 0.005$) values and GS. It seems that lower lipid acyl chain mobility, which could imply more hydrophobic interactions, could correlated significantly with greater puncture force and gel strength of chia oil EGs. Similar observations have been reported in the literature both for conventional oil-in-water emulsions and for EGs, where lipid structure and interactions seem to decisively affect textural properties (Herrero et al., 2011a; Herrero et al., 2011b; Niu et al., 2016; Pintado et al., 2015). It has been suggested that the order/disorder of the lipid chain, which is related to lipid interactions and emulsion droplet size, could be decisive for the textural properties of olive oil emulsion gels stabilized with chia (Pintado et al., 2015).

On the other hand, previous studies showed different rheological properties and morphological differences in the organization of the network structure as functions of the system (protein and/or gelling agents) used to stabilize oil-in-water emulsions. For instance, the presence of a cold gelling agent as MTG can induce a cold-gelling process in oil-in-water emulsions, resulting in a more compact network structure with numerous small cavities associated with gel-like behaviours (Delgado-Pando et al., 2010).

The Raman spectra of chia oil EGs also featured changes in the stretching band C=C at 1658 cm^{-1} (Fig. 2). The intensity of this band decreased ($p < 0.05$) depending on whether the EG contained oat bran (OEG), SPI (SEG) or chia flour (CEG) (Fig. 2 and Table 3). This may be explained among other factors by an increase in lateral pressure due to an order-induced increase in density near the methyl end of the lipid chain (Herrero et al., 2014; Wong & Mantsch, 1983). Differences observed in textural properties between chia oil EGs (Table 2) could therefore be a consequence of this. In this regard, more lateral pressure resulting from increased density near the methyl end of the lipid chain in OEG and SEG (Table 3) could make for stronger texture in these EGs (Table 2).

4. Conclusion

This study highlights a feasible strategy for development of EGs, formulated with chia oil and structured with a cold gelling agent (alginate) and different plant derivative ingredients (oat bran, SPI or chia flour). These potential fat replacers offer some advantages for food applications in that they contain a variety of healthy bioactive compounds (mainly α-linolenic acid and dietary fibre)

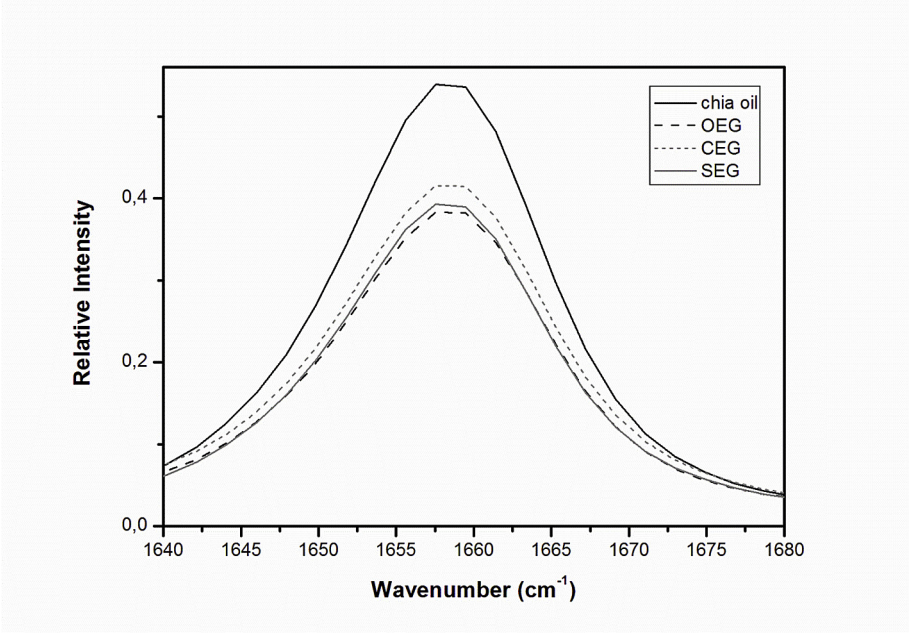

Fig. 2. Raman spectra in the 1640-1680 cm^{-1} region from chia oil (liquid) and chia oil emulsion gels. For samples denomination see Table 1.

and also possess useful technological properties (fluid release, pH, colour and texture).

Differences in the composition of plant ingredients considerably affected both technological (pH, colour, texture, etc.) properties and structural characteristics of chia oil EGs. In this regard, Raman spectroscopy has provided valuable information about the structural state of the network of these chia oil EGs in terms of lipid acyl chain mobility (order/disorder), related to lipid interactions, and of lateral pressure due to higher density near the methyl end of the lipid chain. These lipid structural characteristics in EGs depend on the composition of the plant derivatives (chia flour, oat bran or SPI) used in their formulation. Specifically, chia oil EGs containing oat bran were characterized by lower lipid acyl chain mobility, which could imply more hydrophobic interactions, and more lateral pressure due to greater density near the methyl end of the lipid chain. This particular lipid structural organization of the lipid acyl chain and interactions in oil EGs containing oat was accompanied by a stronger textural feature.

The above considerations suggest that the differences in composition of plant ingredients (oat bran, chia flour or SPI) in the formulation of chia oil EGs could be decisive for mobility (order/disorder), lateral pressure in oil acyl chains or lipid interactions, which appear to be conducive to certain patterns of textural properties. It is thus to be expected that a proper understanding of the connection between a specific lipid acyl structural characteristic and textural behaviour in chia oil EGs will help to select particular EGs for inclusion in a suitable food matrix without this adversely affecting the texture of the end product.

Given their composition, technological properties and lipid structural characteristics, these chia oil EGs could be suitable for novel uses as healthier lipid ingredients in low-calorie and reduced-fat products, for instance to replace animal fats in healthier meat products. The choice of specific chia oil EGs will depend on the properties it is wished to achieve in the food product to which it is added.

On the other hand, complementary studies about the role of proteins and the other components of EGs may be necessary to understand the role of each one in the formation and stabilization of EGs. In this respect, further research may be necessary.

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