**Infrared spectroscopic analysis of structural features and interactions
in olive oil-in-water emulsions stabilized with soy protein**

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Abstract

Lipid and protein structural characteristics of olive oil-in-water-emulsions formulated with various stabilizer systems were investigated using Fourier transform infrared spectroscopy (FT-IR). Proximate composition, water binding and textural properties were also evaluated in these emulsions. Two different olive oil-in-water emulsions were studied: E/SPI prepared with soy protein isolate as a stabilizing system, and E/SPI+SC+MTG prepared with a combination of soy protein isolate, sodium caseinate and microbial transglutaminase as a stabilizing system. Results showed that textural properties ($P<0.05$) were dependent on the stabilizing system. E/SPI+SC+MTG emulsion presented greater ($P<0.05$) lipid chain disorder, more lipid-protein interactions, and more ($P<0.05$) α -helix and β -sheet structures. A relationship between textural and structural properties was also observed as a function of the stabilizing system employed in the formulation of emulsions. A more thorough understanding of this connection could help improve the development of food products with appropriate physical properties.

Keywords: olive oil-in-water emulsion, infrared spectroscopy, lipid structure, protein structure

1. Introduction

A variety of factors in the last ten years have prompted intensified development of healthier meat products (as compared to traditional formulations), particularly with regard to quantitative and qualitative aspects of their lipid profiles. In this respect, various different procedures have been used to incorporate oils, as replacers of animal fat, in the preparation of these meat derivatives. Of these, one that offers numerous advantages is stabilization with pre-emulsions. These technology options for healthier lipid formulations (used in different meat products) were recently the subject of an extensive review (Jiménez-Colmenero, 2007). Of the vegetable oils used in these emulsions, olive oil has received most attention because its intake is associated with a lessened risk of certain diseases (Jiménez-Colmenero, 2007). In particular, it has been indicated that the type of stabilizing system used in olive oil-in-water emulsions which are added as ingredients (animal fat replacers) in healthier meat derivatives could have a relationship with the characteristics of the final product such as texture, water and fat binding properties, microstructure and so on (Jiménez-Colmenero, Herrero, Pintado, Solas & Ruiz-Capillas, 2010).

Stabilization of these emulsions has been assayed with various protein systems (Jiménez-Colmenero, 2007), the most common being caseinate or soy protein isolate. Recently, however, it has been reported that the action of these stabilizing systems is enhanced by incorporation of other components such as meat protein or microbial transglutaminase (Muguruma, et al., 2003; Delgado-Pando, Cofrades, Ruiz-Capillas, Solas & Jiménez-Colmenero, 2010; Herrero, Carmona, Pintado, Jiménez-Colmenero & Ruiz-Capillas, 2011). The behaviour of emulsions is governed by physicochemical and structural properties of the components such as protein and lipids present in the mixture and their interactions with one another (Das & Kinsella, 1990). In general, an oil-in-

water emulsion is defined by three regions: the interior of the lipid droplet, the continuous/aqueous phase, and the interfacial layer. The interfacial layer is a narrow region surrounding each lipid droplet that consists of emulsifiers and/or stabilizers such as proteins or small molecules (monoglycerides, esters or phospholipids) or mixtures of a number of these components, which provide emulsion stability (Das & Kinsella, 1990; Narsimhan, 1992; Dalgleish, 2006). When proteins are used as emulsifiers, because they are amphiphatic they adsorb at the oil-in-water interface and then undergo unfolding and rearrangement to form a stabilizing layer at the droplet surface (Das & Kinsella, 1990; Dickinson, 1998). The properties of interfacial layers depend on their structures (Dalgleish, 2006). The mechanical and rheological properties of the layer are important in the formation and stabilization of food emulsions and vary with the protein type and conditions (Das & Kinsella, 1990). Although much is known about these layers, it is not fully understood how the structure of an interfacial layer affects the properties of an emulsion. In this field of research techniques such as infrared spectroscopic could be a powerful tool.

Infrared spectroscopy, particularly Fourier transform mid-infrared spectroscopy (FT-IR), can be used for structural studies of both lipids and proteins. Its use has several advantages: it is a powerful, rapid, and non-destructive analytical tool capable of providing analytical and structural information on food components such as proteins and lipids and requires minimum sample preparation and relatively small sample amounts (Thygesen, Løkke, Micklander & Engelsen, 2003.). This technique has been used to get structural information for vegetable oils such as soy, olive and sunflower (Guillén & Cabo, 1997a; 1997b, Yang, Irudayaraj & Paradkar, 2005; Sinelli, Cerretani, Di Egidio, Bendini & Casiraghi, 2010). The roles of proteins and lipids in emulsion formation have been shown in oil model systems emulsified with proteins (α -

lactalbumin, β -lactoglobulin) (Fang & Dalgleish, 1997; 1998; Lefèvre & Subirade, 2003; Lee, Lefèvre, Subirade & Paquin, 2009). In a previous study, infrared spectroscopy was used to study emulsions formulated with olive oil and stabilized either with sodium caseinate or with mixtures of sodium caseinate and transglutaminase (Herrero, et al., 2011). This research has shown the potential of this technique as a means of obtaining details of protein secondary structures, chain lipid order and lipid-protein interactions. The use of infrared spectroscopy can thus help understand the behaviour of oil-in-water emulsions prepared with different emulsifying systems and thereby help improve the preparation of foods in which the emulsion ingredients influence their structural and physicochemical properties (e.g. texture, fat and water binding properties).

A clear understanding of the key structural properties of emulsions as determined by the stabilizing systems used will make it easier to choose the one that is most appropriate for the development of oil-in-water emulsions. The aim of this work was therefore to determine the protein and lipid structural characteristics of various olive oil-in-water emulsions stabilized with various emulsifying systems consisting of soy protein isolate and a combination of soy protein isolate, sodium caseinate and microbial transglutaminase, using infrared spectroscopy. These structural characteristics were also associated with physical properties.

2. Materials and methods

2.1. Materials

Soy protein isolate (SPI) (92.1% protein content) and sodium caseinate (SC) (86.4% protein content) were commercial products from Julio Criado Gómez SA (Alcorcón, Spain). Microbial transglutaminase (MTG) ACTIVA WM (Ajinomoto Europe Sales GmbH, Hamburg, Germany) was used. According to the supplier's specifications this enzyme was prepared with a mixture containing 1% transglutaminase and 99% maltodextrin, with a standard transglutaminase activity of approximately 100 units/g. Olive oil (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain) with 13% SFAs, 79% MUFAs and 8% PUFAs was used in all emulsions (Delgado-Pando, et al., 2010). D₂O was used to deuterate the different samples.

2.2. Preparation of stabilizing systems in aqueous solution and olive oil-in-water emulsions.

Different stabilizing systems were prepared in aqueous solution and used as reference: (1) soy protein isolate (SPI) and (2) a mixture of SPI, SC and MTG (SPI+SC+MTG) (Table 1). They were prepared by mixing (in a Stephan Universal Machine UM5, Stephan Machinery GmbH, Hameln, Germany) hot water (60-65 °C) with SPI or SPI, SC and MTG for 2 min (Table 1).

Two different types of olive oil-in-water emulsions were considered: (1) emulsion prepared with SPI as a stabilizing system (E/SPI), and (2) emulsion prepared with a combination of SPI, SC and MTG as a stabilizing system (E/ SPI+SC+MTG) (Table 1).

Olive oil-in-water emulsion with only SPI as a stabilizer (E/SPI) was prepared by mixing (in a Stephan Universal Machine UM5, Stephan Machinery GmbH & Co., Hameln, Germany) hot water (60-65 °C) with SPI for 2 min (Table 1). When the mixture was cold (5°C) olive oil was gradually added with the homogenizer in operation. Once all the oil was added, the mixture was stirred again for 3 min with a control bath at 5 °C (Table 1). The emulsion E/SPI+SC+MTG was prepared in the same way as E/SPI. The only difference was that SPI+SC+MTG instead of only SPI were used in the initial step (Table 1).

Each type of sample was stuffed into metal moulds under pressure to compact them and prevent air bubbles, and stored in a chilling room at 2 °C for 24 h until analysis. Each emulsion was prepared in duplicate.

2.3. Proximate analysis

Sample moisture and ash contents were determined (AOAC, 2000) in triplicate. Protein content was measured in quadruplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). Fat content was evaluated in triplicate according to Bligh and Dyer (1959).

2.4. Water and fat binding properties

Water and fat binding properties were determined (in quadruplicate) by measuring water and fat loss (Jiménez-Colmenero, Carballo & Solas, 1995). The emulsions were stuffed into tubes and hermetically closed then heated in a waterbath for 30 min at 70 °C. They were then opened and left to stand upside down (for 50 min) to release the separated fat and water onto a plate. Total loss was measured as % of initial sample weight. Water loss was determined as % weight loss after heating the total

released fluid (fat and water) for 16 h on a stove at 100°C. Fat loss was calculated as the difference between total loss and water loss.

2.5. Penetration tests

Samples were tested at room temperature (22 °C). The test was performed using a 6 mm diameter cylindrical stainless steel plunger attached to a 5 kg load cell connected to the crosshead of a TA.XTplus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). Force-time curves were obtained at 0.8 mm/s crosshead speed and analysed. The rheological parameters of each sample derived from these curves were: (a) penetration force (PF, N), the force exerted at 10 mm in E/SPI because there was no breaking point, and at the point of gel rupture in E/SPI+SC+MTG; (b) gel strength (GS, J), which is defined as the area enclosed by the force-deformation curve at 10 mm in E/SPI and at the point of gel rupture in E/SPI+SC+MTG. Six determinations were carried out per sample.

2.6. Infrared spectroscopy (FT-IR)

2.6.1. Sample preparation

Protein structure information was acquired through the amide I band (1700-1600 cm^{-1}). However, samples prepared with H_2O exhibit strong absorption of water with a maximum between 1650 and 1640 cm^{-1} (Surewicz & Mantsch, 1996). Therefore, in order to obviate this problem in the protein structure analysis, deuterated olive oil-in-water emulsion samples were prepared. Samples were deuterated according to Herrero et al. (2011). Samples of about 1 mg weight were placed in sealed glass containers having a small glass tube with 1 ml D_2O . In this way samples were exposed to excess D_2O vapour and the consequent isotopic exchange was left to carry on for 4 days at

about 2°C. Deuteration was monitored by means of the OH stretching band. This procedure was carried out in triplicate for each type of sample.

Non-deuterated and deuterated stabilizing systems (SPI and SPI+SC+MTG) in aqueous solution were prepared as references.

Non-deuterated emulsion samples were used to study structural modifications of lipids (3050-2800 cm^{-1}). Olive oil was analysed as reference for that purpose.

2.6.2. *Spectroscopic measurements*

All spectra were collected using a PerkinElmer 1725X spectrometer equipped with a high sensitivity deuterated triglycine sulphate detector at a spectral resolution of 2 cm^{-1} over a range of 4000–800 cm^{-1} . Thin solid films for subsequent spectroscopic measurement were obtained by pressing ~1 mg of sample between two CaF_2 windows (Herrero et al., 2010). These windows were also used for spectral measurement of liquid samples corresponding to solutions of emulsifying systems. The spectra resulted from accumulation of 48 scans which were carried out at a rate of 0.5 cm s^{-1} . Measurements were performed on three samples for each type of emulsion, stabilizing system in aqueous solution and olive oil. Three different portions were scanned for each sample. The spectra were summed to give final FT-IR spectra of 144 scans per sample. These were used as background spectra for subsequent conversion to absorbance spectra and data analysis. A two-point baseline correction was made by drawing a baseline connecting two selected points located near 3600 and 3480 cm^{-1} . A total of three sum spectra were analysed per type of emulsion, stabilizing system in aqueous solution and olive oil.

The half-bandwidths of the 2925 and 2854 cm^{-1} bands were measured as follows. A straight line was drawn as a baseline tangentially between the absorbance

minima occurring on either side of the band in question (near 2882 and 2990 cm^{-1} for the 2925 cm^{-1} band, and 2810 and 2882 cm^{-1} for the 2854 cm^{-1} band). The half-bandwidth for each band was calculated by measuring the bandwidth half-way between the band intensity maximum and the corresponding baseline.

2.6.3. Data analysis

Spectral data were treated with the Grams/AI (Thermo Electron Corporation, Waltham, MA) software, which includes baseline correction, smoothing, solvent subtraction and curve-fitting.

The spectral contributions from residual water vapour were subtracted as appropriate using a set of water vapour spectra recorded under the same conditions. The resulting difference spectra were subsequently smoothed with a nine-point Savitsky-Golay function to reduce the noise.

In order to eliminate any spectral influence of water in the 3000-2800 cm^{-1} vCH region of the emulsion samples, the spectral contribution of water in the 3600-3100 cm^{-1} range was subtracted as appropriate from emulsion spectra using the 2125 cm^{-1} association band of water as an internal intensity standard (Lavialle, Adams, & Levin 1982; Vincent, Steer, & Levin, 1984). The water-free spectra of the corresponding aqueous emulsifying system (SPI or SPI+SC+MTG) were then subtracted, based on the elimination of the amide II band using a subtraction factor so that the intensity peak near 1545 cm^{-1} is not visible. The half-bandwidths of the 2925 ($\nu_{\text{as}}\text{CH}_2$) and 2854 ($\nu_{\text{s}}\text{CH}_2$) cm^{-1} bands were measured in the resulting difference spectra.

Protein structure was analysed in deuterated samples (treated with D_2O) by fitting the 1700–1600 cm^{-1} amide I region to a sum of Lorentzian band components with a nonlinear least-squares procedure. The mathematical solution to this decomposition

may not be unique, but if restrictions are imposed such as maintenance of the initial band positions in an interval of $\pm 1 \text{ cm}^{-1}$, exclusion of bands with negative heights, keeping the bandwidth within the expected limits or agreement with theoretical boundaries or predictions, the result becomes unique in practice. The number and position of the bands were obtained from deconvoluted spectra. The content of the various secondary structure elements was estimated by dividing the integral intensity of one amide I band component by the total intensity of all amide I band components. On the basis of literature references, the bands appearing near 1682 cm^{-1} are attributed to β -sheet structure, and those in the $1660\text{-}1650 \text{ cm}^{-1}$ and $1640\text{-}1618 \text{ cm}^{-1}$ ranges are attributed to α -helix and β -sheet structures respectively (Kalinin, Baikalov, & Venyaminov, 1990; Dong et al., 1996; Barth, 2007). The amount of each secondary structure element is given in percentage terms.

2.7. Statistical analysis

Analysis of variance (ANOVA one-way) and Tukey's multiple range test were performed in order to evaluate the statistical significance ($P < 0.05$) of the effect of olive oil-in-water emulsion formulation. The normal distribution of samples was checked using the Shapiro–Wilks test. The Kruskal-Wallis test was used to test samples that did not fit the normal distribution. Statistical analysis was performed using Statgraphics Plus version 5.0.

3. Results and discussion

3.1. Proximate analysis and water and fat binding properties.

In general there were few differences in the proximate analyses of the samples, and these were consistent with product formulation. The moisture and ash percentages

were around 43 % and 0.3 % respectively in olive oil-in-water emulsions. The protein contents of emulsions E/SPI and E/SPI+SC+MTG were 3.5 % and 4.6 % respectively. Fat content of emulsions was around 53 %. These results are consistent with product formulation (Table 1).

There was no noticeable release of exudates in olive oil-in-water emulsions after heating. These results showed that the water and fat binding properties of the different olive oil-in-water emulsions were optimal. Similar results have been reported in other olive oil-in-water emulsions prepared with sodium caseinate as an emulsifying system (Herrero et al., 2011), and oil (healthier lipid combination)-in-water emulsions prepared with different protein systems (Delgado-Pando, et al., 2010). The excellent water and fat binding properties suggest that oil-in-water emulsions could be an excellent choice, for example, for use as animal fat replacers in the formulation of healthier lipid meat products.

3.2. Penetration test.

Textural behaviour of olive oil-in-water emulsions depends on the stabilizing system used in their formulation (Fig. 1). E/SPI emulsion behaved like a viscous material and lacked a gel structure given that there was no breaking point, and penetration force (PF) and gel strength (GS) values were the lower ($P < 0.05$). E/SPI+SC+MTG emulsion presented different textural properties, in terms of PF and GS (Fig. 1), and a breaking point was observed, indicating a gel structure. These results are consistent with previous data obtained from oil-in-water emulsions in terms of textural parameters such as penetration force and gel strength (Delgado-Pando, et al., 2010; Jiménez-Colmenero et al., 2010; Herrero et al., 2011). The emulsifying system used in this olive oil-in-water emulsion contained a mixture of components (SPI, SC and MTG) the most abundant of which was (Table 1). This emulsifying system

produced the highest values of PF and GS (Fig. 1). These textural properties could be related to MTG, which can interact with SC and SPI, thereby helping stabilize emulsions (Lee, Choi & Moon, 2006). MTG has been reported to catalyse protein cross-linking (Kurth & Roger, 1984; Muguruma et al., 2003), which would help to explain why this type of emulsion was stiffer (Fig. 1).

3.3. Infrared spectroscopic analysis

The structural properties of the olive oil-in-water emulsion studied in the present work were determined using infrared spectroscopy to examine their principal components: lipids and proteins.

3.3.1. Lipid structure.

Fig. 2 shows a typical FT-IR spectrum in the 3050-2800 cm^{-1} region of pure olive oil and olive-oil-in-water emulsions (E/SPI and E/SPI+SC+MTG). Several bands are visible in this infrared region. A weak band near 3005 cm^{-1} at the higher wavenumber side of this region is generated by the *cis* double-bond CH stretching vibration ($\nu_{\text{CH=}}$). The methyl symmetrical and asymmetrical stretching vibration ($\nu_{\text{s}}\text{CH}_3$ and $\nu_{\text{as}}\text{CH}_3$) causes shoulders at approximately 2954 and 2870 cm^{-1} respectively. Those bands did not differ between the oil-in-water emulsions studied. In addition, two strong bands at 2925 and 2854 cm^{-1} are predominant in all spectra (Fig. 2). These are due respectively to the asymmetric and symmetric stretching vibrations of the acyl CH_2 groups (Guillen, & Cabo, 1997a; 1997b, van de Voort, Sedman & Russin, 2001). Broadening of the spectral profile of these bands is generally attributed to diminishment of the conformational order of the lipid acyl chains and increasing of their dynamics (Snyder, Strauss & Elliger 1982; Casal & Mantsch, 1984; Fraile, Patrón-Gallardo,

López-Rodríguez & Carmona, 1999). A broadening of the 2925 cm^{-1} ($\nu_{\text{as}}\text{CH}_2$) and 2854 cm^{-1} ($\nu_{\text{s}}\text{CH}_2$) bands upon formation of olive oil-in-water emulsion was clearly visible (Fig. 2). Similar results have been reported in olive oil-in-water emulsions prepared with sodium caseinate and sodium caseinate and MTG as emulsifying systems (Herrero et al., 2011). Quantification of the intensity of the 2925 cm^{-1} ($\nu_{\text{as}}\text{CH}_2$) and 2854 cm^{-1} ($\nu_{\text{s}}\text{CH}_2$) bands confirmed a significant increase in half-bandwidth values of all oil-in-water emulsions when compared with pure olive oil (Table 2). The significant broadening of these bands may be ascribed to the fact that protein chains are inserted to some extent between the acyl chains of the oil. Proteins are composed of hydrophobic and hydrophilic polypeptide side chains (Das & Kinsella, 1990). The hydrophobic side chains are preferentially located at least partly within the oil phase, whereas the hydrophilic side chains tend to be located towards the aqueous side of the interface. This preferential orientation of hydrophobic side chains in the oil involves interactions between structurally dissimilar acyl and protein chains, which cause disordering of the olive oil lipid chain upon addition of the corresponding emulsion/stabilizer. The E/SPI+SC+MTG emulsion in particular showed the highest ($P<0.05$) half-bandwidth values of the 2925 and 2854 cm^{-1} bands (Fig. 2 and Table 2) and hence the greatest lipid chain disorder or lipid-protein interactions. Lipid chain disorder could be due to augmented lipid-protein interaction promoted by the particular protein structure as a result of the action of MTG, which generates protein crosslinking between polar side chains (glutamine, lysine, arginine) (Kuraishi, Yamazaki & Yasuyuki, 2001; Yokoyama, Nio & Kikuchi, 2004), accompanied by exposure of side chain hydrophobic groups to the corresponding protein surface. This situation obviously promotes insertion of these hydrophobic groups into the oil droplets and subsequent lipid chain disordering or lipid-protein interactions and band broadening.

3.3.2. Protein structure.

The infrared region around 1700–1600 cm^{-1} is the one most often used to extract information on protein secondary structure (Barth, 2007). This spectral region is dominated by a strong band, called the amide I band, which represents primarily the C=O stretching vibrations of the amide groups (coupled to in-plane bending of the N-H and stretching of the C-N bonds) (Surewicz & Mantsch, 1996). The spectral profile of the amide I band was used in the present study to determine protein structural changes occurring upon formation of the oil-in-water emulsion. Comparison of deuterated emulsifier systems in aqueous solution (SPI and SPI+SC+MTG) and olive oil-in-water emulsion showed a shift of the absorption maximum frequency from 1630 to 1641 cm^{-1} in E/SPI (Fig. 3) and from 1631 to 1653 cm^{-1} in E/SPI+SC+MTG (Fig. 4), which is indicative of protein structural changes upon emulsion formation. Similarly, other infrared spectroscopy studies have shown a shift in the amide I band to higher frequencies when proteins participate in oil-in-water emulsion (Lee, Lefèvre, Subirade, & Paquin, 2007, Herrero et al., 2011). This spectral result was interpreted to mean that protein secondary structures changed in the sense that they were more helically ordered in emulsion than in solution, particularly after adsorption in oil/water interfaces (Lee et al., 2007). Once at the interface, most proteins unfold to varying extents and reorient, rearrange, and spread to form a continuous cohesive interface layer. The hydrophobic side chains orient in the apolar oil phase while polar charged segments extend into the aqueous phase, but most of the molecule occupies the interface, interacts with neighbouring molecules, and imparts strength and viscosity to the film (Das & Kinsella, 1990). All this would explain the consequent alterations of secondary protein structure as revealed by the amide I band frequency upshift upon emulsion formation (Figs. 3 and

4). This spectral change has been attributed to α -helix structure enrichment (Jackson & Mantsch 1992; Barth, 2007).

In order to extract more precise information on protein secondary structure, in terms of contents of α -helix, β -sheet and unordered structure components, there is a more practical approach based on deconvolution of the amide I region (1700–1600 cm^{-1}), whereby this band can be further resolved. The bands obtained after deconvolution were assigned on the basis of literature references (Kalnin et al., 1990; Barth, 2007). Protein secondary structure contents were significantly different depending on the stabilizing system used in the preparation of each emulsion. In E/SPI emulsions, the deconvoluted spectrum in the amide I region exhibits bands that are attributed to 19.0% α -helix, 31.6% β -sheet and 49.4 % unordered structure (Fig. 5). This means an increase of about 5% α -helix and 11% unordered backbone and a decrease of about 17% of β -sheet structure in olive oil-in-water emulsion (E/SPI) when compared with SPI in heavy water solution (deuterated). Many, if not all, adsorbed proteins exist in conformations which are different from their native states, although for globular proteins like SPI, the change in secondary structure may be limited. This is a result of the tendency of hydrophobic parts of the molecules to be adsorbed to the hydrophobic interface, with a consequent distortion of their secondary or tertiary structures (Dalgleish, 2006). Soy proteins present a globular structure and consist of two major components: β -conglycinin and glycinin (García, Torre, Marina & Laborda, 1997, Herrero, Jiménez-Colmenero & Carmona, 2009). In other globular proteins such as whey proteins (β -lactoglobulin) the partial unfolding of the globular protein structure following adsorption in the interface layer causes exposure of the reactive sulphydryl group, leading to slow polymerization of the adsorbed protein via sulphydryl-disulphide interchange (Dickinson & Matsumura, 1991; McClements, Monahan & Kinsella, 1993;

Fang & Dalgleish, 1998; Dickinson, 1998). This could account for the stronger textural properties of emulsions formulated with soy protein as a stabilizing system (E/SPI) when compared with other olive oil-in-water emulsions prepared with sodium caseinate as an emulsifying system (Herrero et al., 2011). Experimental observation of interfacial viscosity of adsorbed proteins have shown that globular proteins like soy protein provide much better mechanical properties than flexible random coil proteins like β -casein (Narsimhan, 1992). In addition, globular proteins like SPI have a three-dimensional structure in which the hydrophobic amino acid side chains are distributed between the surface and the interior of the protein molecule (Das & Kinsella, 1990). Therefore, if a protein has a large number of hydrophobic amino acids on the surface (depending on their disposition), greater interaction with the oil surface is possible, leading to a better emulsion (Das & Kinsella, 1990).

In E/SPI+SC+MTG emulsion the deconvoluted spectrum in the amide I region revealed 24.0% of α -helix, 39.4% of β -sheet and 36.6 % unordered structure (Fig. 5). When compared with SC+MTG+SPI in aqueous solution, mainly 7% α -helix and 5% unordered structure enrichments were detected.

In both the emulsions studied here, (E/SPI and E/SPI+SC+MTG) secondary protein structural changes were observed after adsorption in oil/water interfaces in terms of an increase of the α -helix structure upon emulsion formation. This structural change is analogous to that occurring in other emulsions prepared with whey protein as an emulsifying system (Lee et al., 2007). However, the α -helix and β -sheet contents were higher in E/SPI+SC+MTG than in E/SPI. The emulsifying system used in E/SPI+SC+MTG contains a mixture of protein (SC and SPI) and MTG. In such a case, there could be competition between emulsifiers to occupy the interface and interact in the layer (Das & Kinsella, 1990). Therefore the interface may be covered by more than

one emulsifier in different amounts. The α -helix and β -sheet enrichments could be promoted by the presence of MTG, which probably induces cross-linking of adsorbed proteins (SPI and SC) in the interface (Faergemand & Murray, 1998). This enzyme catalyses an acyl transfer reaction between the γ -carboxyamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors), including the ϵ -amino group of lysine residues in certain proteins (Kuraishi, Yamazaki & Yasuyuki, 2001; Yokoyama, Nio & Kikuchi, 2004). Crosslinking is most efficient in proteins that contain a glutamine residue in a flexible region of the protein, or within a reverse turn. The flexible caseins are therefore very good substrates (Dickinson, 1997; Gerrard, 2002, Kuraishi et al., 2001). Although globular proteins such as SPI are not attacked by transglutaminase in their native states, the susceptibility of globular proteins to transglutaminase-induced crosslinking may be increased by adsorption at the oil-water interface (Dickinson, 1997), as occurred in E/SPI+SC+MTG emulsion.

Some authors have reported that increased β -sheet content can be explained as due either to interaction among adsorbed protein molecules or to interaction of the protein structure with the lipid phase (Howell, Herman & Li-Chan, 2001). In this connection, the structural results in this study showed that higher α -helix and β -sheet contents in E/SPI+SC+MTG emulsions were accompanied by significantly greater lipid chain disorder or lipid-protein interactions (Fig. 2 and Table 2). The use of a mixture of SPI+SC+MTG as a stabilizing system in the preparation of olive oil-in-water emulsion therefore seems to generate protein crosslinking including β -sheet formation, which is consistent with the interactions with the olive oil interface.

The textural properties of both of the olive oil-in-water emulsions studied here could be related to structural changes observed in proteins and lipids and their interactions. In this regard, E/SPI+SC+MTG presented a gel structure (as evidenced by

increased PF and GS) while E/SPI did not (Fig. 1). The gel structure observed in E/SPI+SC+MTG was accompanied by more α -helix and β -sheet content and greater lipid chain disorder or lipid-protein interactions. This fact could be relevant to textural properties observed in healthier lipid frankfurter formulated with olive oil-in-water emulsions as ingredients (animal fat replacers) prepared with SPI and a combination of SPI, SC and MTG as a stabilizing system (Jiménez-Colmenero et al., 2010). Frankfurters made with an olive oil-in-water emulsion stabilized by a combination of SPI, SC and MTG were firmer than others formulated with olive an oil-in-water emulsion prepared with SPI as an emulsifier.

4. Conclusions

In olive oil-in-water emulsions, infrared spectroscopy shows that protein and lipid structural characteristics depend on the composition of the stabilizing system used. These structural characteristics are accompanied by a specific textural behaviour depending on the emulsifying system used in the preparation of the emulsions.

The formation of the emulsion produces an enriched α -helix structure regardless of the emulsifying system. In particular, the emulsion made with a combination of SPI, SC and MTG exhibited the highest α -helix and β -sheet structure contents, which could be attributed to the role of MTG in protein crosslinking (SPI and SC). In addition, this type of olive oil-in-water emulsion prepared with a combination of SPI, SC and MTG presented the greatest lipid chain disorder and the most lipid-protein interactions. These structural characteristics could be related to their stronger textural properties.

An understanding of the possible relationship between structural and textural properties of olive oil-in-water emulsion formulated with various stabilizing systems could help in choosing the stabilizing systems that are most suitable for the preparation of food products with specific textural characteristics such as healthier lipid meat

products formulated with olive oil-in-water emulsions as ingredients (animal fat replacers).

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Figure captions

Fig. 1. Penetration test parameters, penetration force (■) and gel strength (■), of olive oil-in-water emulsions described in Table 1. Different letters (a, b) indicate significant ($P<0.05$) differences in penetration force, and different letters (x, y) indicate significant ($P<0.05$) differences in gel strength.

Fig. 2. FT-IR spectra in the $3050\text{--}2800\text{ cm}^{-1}$ region of: (a) pure olive oil, (b) olive oil-in-water emulsions prepared with soy protein isolate as a stabilizing system (E/SPI), and (c) olive oil-in-water emulsions prepared with a combination of soy protein isolate, sodium caseinate and transglutaminase (E/SPI+SC+MTG) as a stabilizing system.

Fig. 3. FT-IR spectra in the $1700\text{--}1600\text{ cm}^{-1}$ region of deuterated solution containing soy protein isolate used as reference (SPI) and olive oil-in-water emulsion prepared with a SPI stabilizing system (E/SPI).

Fig. 4. FT-IR spectra in the $1700\text{--}1600\text{ cm}^{-1}$ region of deuterated solution containing soy protein isolate, sodium caseinate and transglutaminase used as reference (SPI+SC+MTG) and SPI+SC+MTG olive oil-in-water emulsion (E/SPI+SC+MTG).

Fig. 5. Percentages of protein secondary structure, α -helix (■), β -sheet (■) and unordered (□) structure, of olive oil-in-water emulsions prepared with soy protein isolate (E/SPI) and a combination of soy protein isolate, sodium caseinate and transglutaminase (E/SPI+SC+MTG) as a stabilizing system. Different letters indicate significant ($P<0.05$) differences in α -helix (a, b), β -sheet (x, y) and unordered (m, n) content.

Fig. 1

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Figure 2

Figure 3

Figure 4

Fig. 5

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Table 1. Formulation (g) of different stabilizing systems (in aqueous solution) and olive oil-in-water emulsions.

	SPI	SC	MTG	Water	Olive oil
Stabilizing systems					
SPI	52.6	-	-	947.3	-
SPI+SC+MTG	52.0	9.0	3.0	936.0	-
Emulsions					
E/SPI	52.6	-	-	421.0	526.3
E/SPI+SC+MTG	52.0	9.0	3.0	416.0	520.0

E: olive oil-in-water emulsion; SPI: soy protein isolate; SC: sodium caseinate; MTG: microbial transglutaminase.

Table 2. Half-bandwidth values of the 2925 ($\nu_{as}CH_2$) and 2854 (ν_sCH_2) cm^{-1} bands of pure olive oil and olive oil-in-water emulsions described in Table 1.

Samples	Half-bandwidth	
	$\nu_{as}CH_2$ (2925 cm^{-1})	ν_sCH_2 (2854 cm^{-1})
Pure olive oil	27.6±0.4 ^a	15.4±0.3 ^a
E/SPI	35.8±0.5 ^b	16.9±0.8 ^b
E/SPI+SC+MTG	37.7±0.4 ^c	17.8±0.2 ^b

E: olive oil-in-water emulsion; SPI: soy protein isolate; SC: sodium caseinate; MTG: microbial transglutaminase. Means ± standard deviation. Different letters in the same row indicate significant differences (P<0.05).