Lipid and protein structure analysis of frankfurters formulated with olive oil-in-water emulsion as animal fat replacer

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Abstract

Lipid and protein structural characteristics of frankfurter formulated with olive oil-in-water emulsion stabilized with soy protein isolate (SPI) as pork backfat replacer were investigated using Fourier transform infrared spectroscopy (FT-IR). Proximate composition and textural properties were also evaluated. Different frankfurters were reformulated: F/PF with pork backfat, F/SPI with oil-in-water emulsion stabilized with SPI and F/SPI+SC+MTG with emulsion stabilized with a combination of SPI, sodium caseinate (SC) and microbial transglutaminase (MTG). Replacement of pork backfat with these emulsions produced an increase ($P<0.05$) of hardness, springiness, cohesiveness and chewiness but a reduction ($P<0.05$) of adhesiveness. F/SPI and F/SPI+SC+MTG frankfurters showed the lowest ($P<0.05$) half-bandwidth in the 2922 cm^{-1} band, which could be related to lipid chains were more ordered than in F/PF. Modifications in the amide I band profile revealed a higher concentration of aggregated intermolecular β -sheets in F/SPI+SC+MTG samples. Lipid and protein structural characteristics could be associated with specific textural properties of healthier frankfurters.

Keywords: lipid structure, protein structure, olive oil-in-water emulsion, soy protein isolate, healthier lipid frankfurter, texture.

1. Introduction

In meat systems, infrared spectroscopy (IR), particularly Fourier Transform Infrared Spectroscopy (FT-IR), can furnish information about the secondary structure of proteins and the structure of lipids (Damez & Clerjon, 2008; Herrero, Carmona, Jiménez-Colmenero, & Ruiz-Capillas, 2010). FT-IR has been used in meat matrixes to create models that can predict sensory characteristics and technological properties, and also to study structural changes occurring in proteins during processing (Kirschner, Ofstad, Skarpeid, Høst & Kohler, 2004; Böcker, Ofstad, Bertram, & Egeland, 2008; Damez & Clerjon, 2008; Herrero et al, 2010).

It is important to note that there are also a number of studies reporting the use of infrared spectroscopy to obtain structural information in vegetable oils (Guillén & Cabo, 1998, Yang, Irudayaraj, & Paradkar, 2005) and to determine composition and degree of lipid unsaturation in adipose and fatty tissue of different kinds of meat (Guillén & Cabo, 1998; Olsen, Rukke, Flatten, & Isaksson, 2007;). These studies show that spectral results correlate significantly with results obtained through other methods commonly used to determine fatty acid profiles. The role of proteins and lipids in emulsion formation has been demonstrated in model systems of oils emulsified with proteins (α -lactalbumin, β -lactoglobulin) (Fang & Dalgleish, 1998; Lee, Lefèvre, Subirade, & Paquin, 2009). Preliminary IR spectroscopy assays have been conducted at our laboratory on olive oil-in water emulsions stabilized with various protein systems. The spectral results showed the influence of the stabilizing system on the structural characteristics of these oil-in-water emulsions (Herrero, Carmona, Pintado, Jiménez-Colmenero, & Ruiz-Capillas 2011a; 2011b).

In recent years there has been growing consumer interest in healthier meat derivatives in which animal fat is replaced by other vegetable and/or marine oil sources

more in line with nutritional recommendations. Of the procedures used to incorporate oils as ingredients in the manufacture of meat derivatives, stabilization with oil-in-water emulsions offers numerous advantages (Jiménez-Colmenero, 2007). Various procedures have been reported to make pre-emulsions for addition to meat products (Jiménez Colmenero, 2007, Cáceres et al., 2008). The most commonly employed are ones in which protein systems like caseinate or soy protein isolate are used, although recently it has been suggested that their activity could be assisted by means of other components such as meat protein or microbial transglutaminase (Muguruma, Tsuruoka, Katayama, Erwanto, Kawahara, & Yamauchi, 2003; Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, 2010). In such cases, the use of these emulsions as animal fat replacers in meat derivatives will influence the quality attributes of the product in different ways. In this respect, studies dealing with the reformulation of healthy meat products have focused mainly on aspects relating to changes in sensory characteristics and technological properties, as these are the quality characteristics most appreciated by consumers and they help to determine the commercial success or failure of any new meat derivative. However, in order to develop better products of this kind we need to gain a clearer understanding of the complex relationship between the structure of their components and many of their quality-related (technological, sensory, etc.) properties.

Within this line of action, preliminary studies have been carried out on the viability of IR spectroscopy to analyse the structural characteristics of healthier meat derivatives formulated with olive oil-in-water emulsion stabilized with casein as a pork backfat replacer (Carmona, Ruiz-Capillas, Jiménez-Colmenero, Pintado, & Herrero, 2012). These studies have highlighted some interesting possibilities that this technique can offer in terms of changes in protein secondary structure and changes in lipid chain order, or determination of lipid-protein interactions. These findings underline the

importance of the stabilizing system used in the formulation of the oil-in-water emulsion, which will influence both the technological properties and the structural characteristics of the reformulated meat product.

In this connection there is clearly a need to gain a better understanding of the role played by different oil-in-water emulsion in both the technological properties and the structural characteristics of the reformulated meat product. IR spectroscopy offers a powerful tool with which to examine in depth the molecular changes that occur in both lipids and proteins. The aim of this work was therefore to use FT-IR to elucidate lipid and protein structural features of frankfurters formulated with an olive oil-in-water emulsion stabilized with soy protein as a pork backfat replacer. A further aim was also to establish possible connections between structural features and such technological properties as texture of the final healthier meat product.

2. Materials and methods

2.1. Materials

Fresh post-rigor pork meat (mixture of *M. biceps femoris*, *M. semimembranosus*, *M. semitendinosus*, *M. gracilis* and *M. adductor*) and pork backfat were obtained from a local meat market. The meat was trimmed of fat and connective tissue and the pork fat was passed through a grinder with a 0.4 mm plate. Lots of approx. 1 kg were vacuum packed, frozen and stored at -20 °C until use, which took place within 2 weeks.

Ingredients used for preparation of oil-in-water emulsions included: olive oil (13 % SFA, 79 % MUFA and 8 % PUFA) (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain); soy protein isolate (SPI) (92.1% protein content) (Trades SA, Barcelona, Spain); sodium caseinate (SC) 86.4 % protein content (Julio Criado Gómez SA, Alcorcón, Spain) and microbial transglutaminase (MTG) (ACTIVA WM, Ajinomoto Europe Sales GmbH, Hamburg, Germany). According to supplier information, the enzyme was used in a mixture containing 1 % transglutaminase and 99 % maltodextrin, with a standard transglutaminase activity of approximately 100 units/g.

Other ingredients and additives used were sodium chloride (Panreac Química, S.A. Barcelona, Spain), sodium tripolyphosphate (STP) (Manuel Riesgo, S.A. Madrid, Spain) and sodium nitrite (Fulka Chemie GmbH, Buchs, Germany).

2.2. Preparation of olive oil-in-water emulsions.

Two different types of olive oil-in-water emulsions were considered: (1) emulsion prepared with SPI as a stabilizing system (O/SPI), and (2) emulsion prepared with a combination of SPI, SC and MTG as a stabilizing system (O/ SPI+SC+MTG). These olive oil-in-water emulsions were prepared according to Herrero et al. (2011b)

using a homogenizer at 1500 rpm (Stephan Universal Machine UM5, Stephan Machinery GmbH & Co., Hameln, Germany) with a control bath at 5 °C. The O/SPI emulsion was prepared by mixing (in the Stephan Universal Machine) eight parts of water with one part of SPI for 2 min. The mixture was emulsified with 10 parts of olive oil for another 3 min. The concentration of SPI in the emulsion was 5.2%. O/SPI+SC+MTG oil-in-water emulsion was prepared in the same way as O/SPI but including SC and MTG in the initial step. The concentration of SC and MTG in the emulsion was 0.9% and 0.3%, respectively. Percentages were expressed as w/w.

The temperature of the emulsions was less than 10°C at the end of the process. Each type of sample was stuffed into metal moulds of 2 kg capacity under manual pressure to compact them and avoid the air bubbles, and stored in a chilling room at 3 °C for 24 h until analysis. Each emulsion was prepared in duplicate. These oil-in-water emulsions were used as pork backfat replacers in the reformulation of the frankfurters.

2.3. Preparation of frankfurters.

Meat and backfat were thawed (approx. 18 h at 3 ± 2 °C) prior to use. Three different frankfurters were prepared according to (Carmona et al., 2012) as reported in Table 1. Briefly, raw meat material was homogenized and ground for 1 min in a chilled cutter (2 °C) (Stephan Universal Machine UM5, Stephan Machinery GmbH & Co., Hameln, Germany). Half of the pork backfat or oil-in-water emulsion (depending on the formulation), NaCl, STP and sodium nitrite (the last two previously dissolved in the added water) were added to the ground meat and mixed again for 1 min. The rest of the additives, the pork backfat or the olive oil-in-water emulsion as fat replacer, were added and the whole homogenized for 1 min. Finally the whole meat batter was homogenized

under vacuum conditions for 2 min. Mixing time was standardized at 5 min. The final batter temperature was below 14 °C in all cases.

The meat batter was manually stuffed into 20 mm diameter Nojax cellulose casings (Viscase S.A., Bagnold Cedex, France) and hand-linked. Frankfurters were heat processed in an Eller smokehouse (model Unimatic 1000, Micro 40, Eller, Merano, Italy) until the core of the product reached 70 °C. The heat processing conditions were defined beforehand: in the definition process the internal temperature was monitored throughout heating by means thermocouples inserted in various sausages at the product's thermal centre and connected to a temperature recorder (Yokogawa Hokuskin Electric YEM, Mod. 3087, Tokyo, Japan). Once heating was complete, the frankfurters were cooled (at room temperature), packed without vacuum and placed in a cold chamber (2 °C) until analysis. Each frankfurter formulation was prepared in duplicate and for each analysis different frankfurters were analyzed.

2.4. Proximate analysis.

Moisture and ash contents of the frankfurters were determined in triplicate (AOAC 2000). 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 (Bligh & Dyer, 1959).

2.5. Texture Profile Analysis.

Texture Profile Analysis (TPA) was performed in a TA.XTplus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Six cylindrical frankfurter cores (diam = 20 mm, height = 20 mm) from each formulation were axially compressed to 40% of their original height (Bourne 1978) with an aluminium cylinder probe P/25. Force-time

deformation curves were obtained with a 5 kg load cell, applied at a crosshead speed of 1 mm/sec. Attributes were calculated with Texture Expert program as follows: hardness (Hd) = peak force (N) required for first compression; cohesiveness (Ch) = ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp) = distance (mm) the sample recovers after the first compression; adhesiveness (Ad) = area under the abscissa after the first compression (Nxs). Measurement of samples was carried out at room temperature.

2.6. Infrared spectroscopy (FT-IR)

2.6.1. Spectroscopic measurements. Thin solid films for subsequent spectroscopic measurement were obtained by pressing approximately 1 mg of solid samples (frankfurter) between two CaF₂ windows (Carmona et al., 2012). 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. The spectra resulted from accumulation of 48 scans which were carried out at a scan speed of 0.5 cm s⁻¹. Measurements were performed on three samples. Three different portions were recorded for each sample. The spectra were summed, giving in final FT-IR spectra of 144 scans per sample. These sum spectra were used as background spectra for subsequent conversion to absorbance spectra and data analysis. A total of three sum spectra were analysed per sample.

The spectral contributions from residual water vapour were appropriately subtracted using a set of water vapour spectra recorded using an infrared gas cell having 2 cm pathlength under the same conditions. The resulting difference spectra were subsequently smoothed with a nine-point Savitsky-Golay function to reduce the noise.

Spectral data were treated with the Grams/AI (Thermo Fisher Scientific Inc., Billerica, MA, USA) software version 9.00 R2, which includes baseline correction, smoothing, solvent subtraction and curve-fitting.

2.6.2. Lipid structure analysis. Spectral region 3000-2800 cm^{-1} was analyzed to study lipid structure. In order to eliminate any spectral influence of water from the samples in this spectral region (3000-2800 cm^{-1}), the spectral contribution of water was appropriately subtracted from sample spectra using the 2125 cm^{-1} association band of water as an internal intensity standard (Carmona et al., 2012). The water-free spectra of the frankfurter were then subtracted, based on the elimination of the amide II band using a subtraction factor so that the intensity maximum 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 as follows in the resulting difference spectra.

A straight line was drawn as a baseline tangentially between the absorbance minima located on either side of the band in question (near 2990 and 2882 cm^{-1} for the 2925 cm^{-1} band, 2882 and 2810 cm^{-1} for the 2854 cm^{-1} band). The half-bandwidths for each band are calculated by measuring the bandwidth at half height between the band intensity maximum and the corresponding baseline.

2.6.3. Protein structure analysis. Spectral region 1700-1600 cm^{-1} was analyzed to study protein structure. Cooked meat products like frankfurters are characterized by high water content. Water exhibits strong absorption with a maximum located between 1650 and 1640 cm^{-1} (Herrero et al., 2010). This influences the protein structure information which is determined through the amide I band (1700-1600 cm^{-1}) (Herrero et al., 2010). Frankfurter samples were therefore deuterated to avoid this drawback in the protein structure analysis. For deuteration, samples of about 1 mg weight were placed in small glass tubes with an open side, which were placed in sealed glass containers with 1

ml D₂O. In this way samples were exposed to excess D₂O vapour and the consequent isotopic exchange was left to carry on for 4 days at about 2 °C. Deuteration was checked through disappearance of the water νOH band intensity (3600-3000 cm⁻¹ range). This procedure was carried out in triplicate for each type of sample. The use of D₂O influences amide I and II bands. According to well known spectrum-structure correlations (Barth, 2007), amide I band components of α-helical and unordered structures downshift a few wavenumbers; however, the amide II vibrational mode shifts from the 1560-1520 cm⁻¹ region to the 1460-1420 cm⁻¹ region.

Protein structure was quantified in deuterated samples (treated with D₂O) by fitting the 1700–1600 cm⁻¹ amide I region to a sum of Lorentzian band components with a nonlinear least-squares procedure. First, one of the three band parameters (frequency, intensity, halfbandwidth) was fitted after fixing the other two ones; second, two of these were fitted after fixing the remaining parameter; and finally the three parameters were let free for subsequent fitting. The mathematical solution to curve fitting may not be unique, but if restrictions are imposed such as maintenance of the initial band positions in an interval of ±1 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. Fig. 1 showed a typical spectrum of a frankfurter formulated with oil-in-water emulsions stabilized with a mixture of SPI, SC and MTG (F/SPI+SC+MTG) and the Lorentzian amide I band components. The number of Lorentzian amide I band components was dependent on the components of the frankfurter. The number and position of the bands were obtained either from deconvoluted or second derivative spectra. The deconvolution procedure was carried out using an enhancement resolution factor of $k = 2$, and the second derivative spectra were measured through the Savitzky-Golay algorithm by taking 9 points. 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 1660-1650 cm⁻¹ and 1640-1618 cm⁻¹ range are assigned to α -helix and β -sheet structures respectively (Barth, 2007). The differences between samples of each secondary structure element are given in relative 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 the different olive oil-in-water emulsions in the frankfurter 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

Frankfurters presented some differences in proximate analysis which were consistent with product formulation (Table 1). All sausages contained similar ($P > 0.05$) protein levels: 17.30 ± 0.20 , 17.83 ± 0.15 and 18.05 ± 0.29 % for control (F/PF), F/SPI and F/SPI+SC+MTG respectively. Sausages were formulated with the same target muscle protein, so that while the protein in the control frankfurter was from meat raw material (meat and pork backfat), the other samples additionally contained the non-meat proteins used to stabilize the olive oil emulsions (Table 1). There were no differences ($P > 0.05$)

in the moisture content (range from 60.6 ± 0.08 to 61.5 ± 0.15 %) and ash percentages (3.01 ± 0.02 to 3.05 ± 0.03 %) in the different frankfurter formulations. Fat content was generally close to the target level and there were no differences ($P>0.05$) between samples. The corresponding fat contents of F/PF, F/SPI and F/SPI+SC+MTG were 19.40 ± 0.12 , 18.08 ± 0.19 and 18.16 ± 0.40 % respectively. When pork backfat was totally replaced by oil-in-water emulsion (containing 52% olive oil), samples had around 13 g of olive oil per 100 g of product.

3.2. Texture Profile Analysis.

Texture profile analysis parameters were affected by frankfurter formulation (Table 2). Replacement of pork backfat with oil-in-water emulsion generally produced an increase ($P<0.05$) of hardness, springiness, cohesiveness and chewiness. However, frankfurters made with any of the different oil-in-water emulsions presented lower ($P<0.05$) adhesiveness than those made with all pork fat (Table 2). Some authors have observed increased firmness in cooked meat products prepared with pre-emulsified vegetable and/or fish oil as animal fat replacers (Delgado-Pando et al., 2010; Jiménez-Colmenero, Herrero, Pintado, Solas, Ruiz-Capillas, Carmona, 2010; Youssef, & Barbut, 2011; Shao, Zou, Xu, Wu, Zhou, 2011; Carmona et al., 2012). Other authors (Cáceres, E.; García, M. L.; Selgas, 2008) reported that, in general terms, sensory analysis has shown a slight increase in hardness and work of shearing in mortadella (Spanish bologna-type sausage) formulated with higher levels of pre-emulsified fish oil irrespective of the fat content

In the present work, it is important to note that the differences in textural properties between frankfurters (Table 2) are determined mainly by the characteristics of each lipid phase and, probably, its role in the meat protein matrix. Results showed

that F/SPI+SC+MTG (Table 2) presented the highest ($P<0.05$) hardness and chewiness. This textural behaviour seems likely to be a consequence of the role of MTG in stabilizing the emulsion (Delgado-Pando et al., 2010; Herrero et al., 2011b). MTG can interact with SC and SPI, thereby helping stabilize emulsions by promoting protein cross-linking (Lee, Choi & Moon, 2006). Muguruma et al. (2003) reported that texture of chicken sausages was improved (increased breaking stress) by the addition of biopolymers prepared from proteins (soybean protein, casein, whey protein isolate) and transglutaminase. This fact has been attributed to the formation of network structures that contribute to hardness of sausage gels with the addition of biopolymers (Muguruma et al., 2003). In the same way, the addition of MTG to olive oil-in-water probably promotes protein-protein interactions in the emulsion (Herrero et al., 2011a; 2011b; Lee, Choi, & Moon, 2006), which could increase resistance to compression in the final product (F/SPI+SC + MTG).

Previous studies at our laboratory evaluated the influence of various emulsified olive oil stabilizing system on the technological and microstructural properties of the frankfurter (Jiménez-Colmenero et al., 2010; Delgado-Pando et al., 2011). Microstructure studies have shown that the morphology of the reformulated frankfurters is affected by the type of oil-in-water emulsions used in the product formulation. This different microstructural characteristic has been associated with the textural behaviour of reformulated product (Jiménez-Colmenero et al., 2010; Delgado-Pando et al., 2011). Frankfurters formulated with olive oil-in-water emulsion stabilized with a combination of SPI+SC+MTG presented more cavity formation (generally smaller) and greater hardness than frankfurters made with pork backfat (Jiménez-Colmenero et al., 2010; Delgado-Pando et al., 2011). Other authors have reported that meat products prepared with canola oil presented smaller lipid globules and showed greater resistance to

compression than meat products prepared with beef fat, which contained larger fat globules and were less hard (Youssef, & Barbut, 2009, 2010). This fact was attributed to a larger number of small globules present in a given volume and/or a larger surface area covered by proteins, allowing more bonding to the matrix; both can offer more resistance to compression (Youssef, & Barbut, 2009).

The composition and characteristics of the lipid materials, in particular animal fat or olive oil-in-water emulsion, used in the reformulation of frankfurters are important factors determining the textural properties of final cooked meat products. Various factors may contribute to the effect that substituting vegetable oils for animal fat has on the texture of meat products. On the one hand there are those related with the fat source characteristics and their distribution in the protein matrix (Hong, Lee, & Min, 2004; Martín, Ruiz, Kivikari, & Puolanne, 2008). In this connection, previous results in meat batters suggested that different added lipids (soybean oil, pork fat or butter) and thermal treatments induced different changes in textural properties (Shao et al., 2011).

3.3. Infrared spectroscopy (FT-IR)

3.3.1. Lipid structure analysis. Fig. 2 shows typical FT-IR spectra in the 3050-2800 cm^{-1} region of the frankfurters reformulated (F/PF, F/SPI and F/SPI+SC+MTG). These spectra show two predominant strong bands at 2922 and 2852 cm^{-1} which can be assigned to asymmetric and symmetric stretching vibrations of the acyl CH_2 groups respectively (Guillen & Cabo, 1997). Modifications of the half-bandwidth of these bands can be generated by changes in lipid chain order/disorder resulting from protein-lipid interactions (Fraile, Patrón-Gallardo, López-Rodríguez, & Carmona, 1999). Narrowing of the spectral profile of these bands [2922 cm^{-1} ($\nu_{\text{as}}\text{CH}_2$) and 2852 cm^{-1} ($\nu_{\text{s}}\text{CH}_2$)] is generally attributed to increasing conformational order of lipid acyl chains (Fraile, et al.,

1999). The lipid concentration of the reformulated frankfurters from olive oil-in-water emulsion or animal fat was approximately 13-14%, while the concentration from meat endogenous lipids in these products was only 4-5%. Therefore, the lipid concentration of these frankfurters comes mainly from the emulsion or animal fat used in their reformulation. In this connection, the observed changes in lipid structure can be mainly attributed to the incorporation of olive oil in water emulsion in F/SPI and F/SPI+SC+MTG or animal in F/PF. We measured the corresponding half-bandwidths (Fig. 3) to determine the differences between the lipid structures of the reformulated frankfurters in terms of order/disorder of lipid acyl chains. The half-bandwidth values of the 2922 cm^{-1} band were lower ($P<0.05$) when pork backfat was replaced by oil-in-water emulsion (Fig. 3). These results indicate that inter- and intra-molecular lipid order is greater in samples made with olive oil-in-water emulsion, probably due to weaker lipid-protein interactions (Fraile et al., 1999; Herrero et al., 2011a; 2011b). Similar findings on frankfurter lipid structure have been reported in a previous work using olive oil-in-water emulsion stabilized with casein as a pork backfat replacer (Carmona et al., 2012). The lipid concentration of the frankfurters formulated with olive oil-in-water emulsion was 13.5% (data taken of preparation of olive oil-in-water emulsions and Table 1) while the concentration of meat endogenous lipids was only 3.6%. Therefore, the lipid concentration of these frankfurters comes mainly from the emulsion added. In this connection, the observed changes in lipid structure can be attributed to the incorporation of olive oil in water emulsion in the frankfurter formulation. For clarity we included this statement in the manuscript

Fat stabilization in meat batters entails the formation of a thin layer of myofibrillar proteins around the fat globules, so that fat globules are stabilized by

heating forming a gelled protein matrix (gel/emulsion system) (Gordon & Barbut, 1992).

Two components are present in the frankfurter reformulated with oil in-water-emulsion as an animal fat replacer, namely oil-in-water emulsion and meat matrix, where this emulsion has been included. In the preliminary formation of the oil-in-water emulsion, the protein molecules diffuse to and are adsorbed at the oil/water interface and form a continuous cohesive film (Das & Kinsella, 1990). The hydrophobic loops 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 stability to the emulsion (Das & Kinsella, 1990). In a previous work it was reported that the formation of oil in-water-emulsion stabilized with soy protein involves lipid chain disorder or lipid-protein interactions (Herrero et al., 2011b). These processes may limit the capacity of the lipid and protein components of olive oil-in-water emulsion to participate in subsequent lipid-protein interaction in the product's meat matrix. That is consistent with the results of the present work, which indicated more inter- and intra-molecular lipid order (lower lipid-protein interaction) in frankfurters containing emulsion. This would mean that physical entrapment of the lipid phase (oil-in-water emulsion) plays a greater part in the stabilization process of these reformulated products (F/SPI and F/SPI+SC+MTG) (gel contribution). By contrast, the frankfurter formulated with animal fat (F/PF) showed the highest ($P<0.05$) lipid chain disorder (more lipid-protein interaction) (Fig. 2), possibly because meat protein chains can be inserted in major extent between the acyl chains of the animal fat. This would involve more lipid- protein interactions, with the formation of a thin layer of proteins around the fat globules (emulsion theory).

3.3.2. *Protein structure analysis.* The amide I band ($1700-1600\text{ cm}^{-1}$) was used to study the changes occurring in the protein when pork backfat was replaced with olive oil-in-water emulsion in the formulation of the frankfurters (Fig. 4). This band includes 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) (Herrero et al., 2010).

Modification of spectral features (intensity and/or frequency) in the amide I region indicates adjustments associated with secondary structures such as α -helix, β -sheet or unordered. Infrared bands centred between approximately 1650 and 1658 cm^{-1} may be considered characteristics of α -helical structure. Polypeptide backbones in β -sheet conformation give rise to infrared bands between approximately 1620 and 1640 cm^{-1} (Herrero et al., 2010).

Comparison of spectral profiles of the amide I band reveals a shift of the absorption maximum from 1632 to 1620 cm^{-1} in F/SPI+SC+MTG (Fig. 4). This spectral change is indicative of changes in protein structure in terms of greater content of aggregated intermolecular β -sheets (Kirschner et al., 2004; Böcker et al., 2006).

Estimates of secondary structure percentages were obtained by deconvolution of the amide I band region ($1700-1600\text{ cm}^{-1}$). F/SPI+SC+MTG showed a significant ($P<0.05$) increase (28%) of β -sheet structure content attributable to aggregated intermolecular β -sheets in this frankfurter as described above. These results were consistent with literature references reporting that replacement of animal fat with olive oil-in-water emulsion is accompanied by an increase in β -sheet structure (Carmona et al., 2012). Enrichment of aggregated intermolecular β -sheets as observed in the present work could be related to the formation of a denser network providing more firmness in F/SPI+SC+MTG (Table 2). Previous morphological studies have indicated that frankfurter formulated with oil-in-water emulsion stabilized with a mixture of SPI, SC

and MTG presented more cavity formation or narrow particle size distribution, generally due to smaller fat globules (Jiménez-Colmenero et al., 2010; Delgado-Pando et al., 2011). This suggests that there is a larger surface area covered by a denser protein matrix structure; this in turn could be associated with greater aggregation of intermolecular β -sheets (Fig. 3), resulting in firmer products (Table 2). Some authors have shown a positive significant correlation between β -sheet structures and textural properties in meat batters prepared with different lipids (pork fat, soybean oil and dairy butter) (Shao et al., 2011). In particular, meat batters prepared with soybean oil showed greater hardness, springiness, cohesiveness, chewiness and resilience than those made with pork fat or butter. This enhancement of textural properties was accompanied by an increase in β -sheet structures (Shao et al., 2011).

4. Conclusion

Infrared spectroscopy is a useful tool for obtaining direct information on the lipid and protein structure occurring in meat products such as frankfurters as a result of formulation of healthier meat derivatives using olive oil-in-water emulsion stabilized with soy protein as a pork backfat replacer. Changes detected in the lipid structure showed that inter- and intra-molecular lipid order was greater in samples containing olive oil-in-water emulsion as a fat replacer, which suggests that there were less lipid-protein interactions in these meat derivatives. Also, spectral results revealed more aggregated intermolecular β -sheets in frankfurters reformulated with emulsion stabilized with a combination of SPI, SC and MTG. In general, these structural characteristics in both lipids and proteins seem to play an essential role in the textural properties of frankfurter since replacement of pork backfat with oil-in-water emulsion enhanced textural properties in terms of increased hardness, springiness, cohesiveness and chewiness.

To understand structural and textural properties and their possible relationship is important in that it can help to improve reformulation processes for healthier meat derivatives. In this way animal fat can be replaced by oil-in water emulsions which are more in line with health recommendations and whose characteristics better adapt to consumer needs.

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References

- AOAC (2000). Official methods of analysis of AOAC International (17th edition). Maryland, USA: Association of Official Analytical Chemistry.
- Barth, A. (2007). Infrared spectroscopy of proteins. *Biochemical Biophysical Acta*, 1767(9), 1073-1101.
- Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911–917.
- Böcker, U., Ofstad, R., Bertram, H. C., Egelanddal, B., & Kohler, A. (2006). Salt-induced changes in pork myofibrillar tissue investigated by FT-IR microspectroscopy and light microscopy. *Journal of Agricultural and Food Chemistry*, 54(18), 6733-6740.
- Bourne, M. C. (1978). Texture Profile Analysis. *Food Technology*, 32, 62–65.
- Cáceres, E., García, M. L., & Selgas, M. D. (2008). Effect of pre-emulsified fish oil—as source of PUFA n-3 – on microstructure and sensory properties of mortadella, a Spanish bologna-type sausage. *Meat Science*, 80 (2), 183-193.
- Carmona, P., Ruiz-Capillas, C., Jiménez-Colmenero, F., Pintado, T., & Herrero, A. M. (2012). Infrared study of structural characteristics of frankfurter formulated with olive oil-in-water emulsion stabilized with casein as pork backfat replacer. *Journal of Agricultural and Food Chemistry* (in press).
- Damez, J. L., & Clerjon, S. (2008). Meat quality assessment using biophysical methods related to meat structure. *Meat Science*, 80(1), 132-149.
- Das, K. P., & Kinsella, J. E. (1990). Stability of food emulsions: physicochemical role of protein and nonprotein emulsifiers. In J. E. Kinsella, *Advances in food and nutrition research* (pp. 81-201). London: Academic Press, Inc.

492 Delgado-Pando, G., Cofrades, S., Ruiz-Capillas, C., Solas, M.T., & Jiménez-
 493 Colmenero, F. (2010) Healthier lipid combination oil-in-water emulsions
 494 prepared with various protein systems: an approach for development of
 495 functional meat products. *European Journal Lipid Science Technology*, 112(7),
 496 791–801.

497 Fang, Y., & Dalgleish, D. G. (1998). The conformation of α -lactalbumin as a function of
 498 pH, heat treatment and adsorption at hydrophobic surfaces studied by FTIR. *Food*
 499 *Hydrocolloids.*, 12(2), 121–12.

500 Fraile, M. V., Patrón-Gallardo, B., López-Rodríguez, G., Carmona, P. (1999). FT-IR
 501 study of multilamellar lipid dispersions containing cholesteryl linoleate and
 502 dipalmitoylphosphatidylcholine. *Chemistry and Physics of Lipids*, 97(2),
 503 119–128.

504 Gordon, A., & Barbut, S. (1992). Mechanisms of meat batter stabilization: a review.
 505 *Critical Review in Food Science and Nutrition*, 32(4), 299–332.

506 Guillén, M. D., & Cabo, N. (1998). Relationships between the composition of edible
 507 oils and lard and the ratio of the absorbance of specific bands of their Fourier
 508 transform infrared spectra. Role of some bands of the fingerprint region. *Journal*
 509 *of Agricultural and Food Chemistry*, 46(5), 1788–1793.

510 Herrero, A. M., Carmona, P., Jiménez-Colmenero, F., & Ruiz-Capillas, C. (2010).
 511 Applications of vibrational spectroscopy to study protein structural changes in
 512 muscle and meat batter systems. In J. Chalmers, P. Griffiths, E. Li-Chan, (Eds.),
 513 *Applications of vibrational spectroscopy to food science* (pp. 315-328). West
 514 Sussex, UK: John Wiley & Sons.

515 Herrero, A. M., Carmona, P., Pintado, T., Jiménez-Colmenero, F., & Ruíz-Capillas, C.
 516 (2011a). Olive oil-in-water emulsions stabilized with caseinate: Elucidation of

517 protein–lipid interactions by infrared spectroscopy. *Food Hydrocolloids*, 25(1),
 518 12-18.

519 Herrero, A. M., Carmona, P., Pintado, T., Jiménez-Colmenero, F., & Ruíz-Capillas, C.
 520 (2011b). Infrared spectroscopic analysis of structural features and interactions in
 521 olive oil-in-water emulsions stabilized with soy protein. *Food Research*
 522 *International*, 44(1), 360-366.

523 Hong, G., Lee, S., & Min, S. (2004). Effects of replacement pork backfat with soybean
 524 oil on the quality characteristics of spreadable liver sausage. *Food Science and*
 525 *Biotechnology*, 13(1), 51–56.

526 Jiménez-Colmenero, F., Herrero, A., Pintado, T., Solas, M. T., & Ruiz-Capillas, C. (2010).
 527 Influence of emulsified olive oil stabilizing system used for pork backfat
 528 replacement in frankfurters. *Food Research International*, 43(8), 2068-2076.

529 Kirschner, C., Ofstad, R., Skarpeid, H. J., Høst, V., & Kohler, A. (2004). Monitoring of
 530 denaturation processes in aged beef loin by Fourier transform infrared
 531 microspectroscopy. *Journal of Agricultural and Food Chemistry*, 52(12), 3920-
 532 3929.

533 Lee, H. A., Choi, S. J., & Moon, T. W. (2006). Characteristics of sodium caseinate- and
 534 soy protein isolate-stabilized emulsion-gels formed by microbial
 535 transglutaminase. *Journal of Food Science*, 71(6), C352–C357.

536 Lee, S. H., Lefèvre, T., Subirade, M., & Paquin, P. (2009). Effects of ultra-high pressure
 537 homogenization on the properties and structure of interfacial protein layer in whey-
 538 protein-stabilized emulsion. *Food Chemistry*, 113(1), 191–195.

539 Martín, D., Ruiz, J., Kivikari, R., & Puolanne, E. (2008). Partial replacement of pork fat
 540 by conjugated linoleic acid and/or olive oil in liver pâtés: Effect on

physicochemical characteristics and oxidative stability. *Meat Science*, 80(2), 496–504.

Muguruma, M., Tsuruoka, K., Katayama, K., Erwanto, Y., Kawahara, S., Yamauchi, K., Sathe, S. K., & Soeda, T. (2003). Soybean and milk proteins modified by transglutaminase improves chicken sausage texture even at reduced levels of phosphate. *Meat Science*, 63(2), 191-197.

Olsen, E. F., Rukke, E. O., Flatten, A., & Isaksson, T. (2007). Quantitative determination of saturated-, monounsaturated- and polyunsaturated fatty acids in pork adipose tissue with non-destructive Raman spectroscopy. *Meat Science*, 76(4), 628-634.

Shao, J. H., Zou, Y. F., Xu, X. L., Wu, J. Q., & Zhou, G. H. (2011). Evaluation of structural changes in raw and heated meat batters prepared with different lipids using Raman spectroscopy. *Food Research International*, 44(9), 2955-2961.

Yang, H., Irudayaraj, J., & Paradkar, M. M. (2005). Discriminant analysis of edible oils and fats by FTIR, FT-NIR and FT-Raman spectroscopy. *Food Chemistry*, 93(1), 25–32.

Youssef, M. K., & Barbut, S. (2009). Effects of protein level and fat/oil type on emulsion stability, texture, microstructure and color of meat batters. *Meat Science*, 82(2), 228-233.

Youssef, M. K., & Barbut, S. (2010). Physicochemical effects of the lipid phase and protein level on meat emulsion stability, texture, and microstructure. *Journal of Food Science*, 75(2), S108-S114.

Youssef, M. K., & Barbut, S. (2011). Fat reduction in comminuted meat products.effects of beef fat, regular and pre-emulsified canola oil. *Meat Science*, 87(4), 356-360.

Table 1. Formulation (g) of frankfurters elaborated with pork backfat (F/PF) and different olive oil-in-water emulsions (F/SPI and F/SPI+SC+MTG).

Samples	Meat	Pork backfat	Olive oil-in-water emulsion		Water
			O/SPI	O/SPI+SC+MTG	
F/PF	1020	248.7	-	-	196.62
F/SPI	1050	-	375	-	40.32
F/SPI+SC+MTG	1050	-	-	375	40.32

Additives added to all samples: 2.0 g/100 g NaCl; 0.30 g/100 g sodium tripolyphosphate; 0.012 g/100 g sodium nitrite; 0.60 g/100 g flavouring and 0.05 g/100 g of liquid smoke.

F/PF: frankfurter formulated with pork backfat. F/SPI: frankfurter formulated with olive oil-in-water emulsion stabilized with soy protein isolate (O/SPI). F/SPI+SC+MTG: frankfurter formulated with olive oil-in-water emulsion stabilized with a mixture of soy protein isolate, sodium caseinate and microbial transglutaminase (O/SPI+SC+MTG)

Table 2. Textural profile analysis (TPA) of frankfurters formulated with pork backfat (F/PF) and different olive oil-in-water emulsion (F/SPI and F/SPI+SC+MTG).

Samples	Hardness (N)	Springiness (mm)	Cohesiveness (dimensionless)	Adhesiveness (Ns)	Chewiness (Nxmm)
F/PF	38.9 ± 1.6 ^a	7.25 ± 0.03 ^a	0.71 ± 0.02 ^a	-0.56±0.05 ^a	211.6 ± 7.5 ^a
F/SPI	43.8 ± 1.3 ^b	7.52 ± 0.05 ^b	0.77 ± 0.01 ^b	-0.32±0.04 ^b	243.2 ± 9.0 ^b
F/SPI+SC+MTG	54.1 ± 0.9 ^c	7.48 ± 0.07 ^b	0.77 ± 0.01 ^b	-0.33±0.06 ^b	299.1 ± 9.4 ^c

Samples formulated with the corresponding oil-in-water emulsion described in Table 1. Means ± standard desviation. Different letters in the same column indicate significant differences (P<0.05).

FIGURE CAPTIONS

Fig. 1. A typical spectrum of frankfurter formulated with oil-in-water emulsions stabilized with a mixture of soy protein isolated, sodium caseinate and microbial transglutaminase (F/SPI+SC+MTG) and the Lorentzian amide I band components.

Fig. 2. FT-IR spectrum in the $3000\text{-}2800\text{ cm}^{-1}$ region of frankfurter formulated as described in Table 1. A: F/PF; B: F/SPI; C: F/SPI+SC+MTG.

Fig. 3. Half-bandwidth values of the $2922\text{ (v}_{\text{as}}\text{CH}_2)$ and $2852\text{ (v}_{\text{s}}\text{CH}_2)$ cm^{-1} bands of frankfurter formulated with pork backfat (F/PF) and olive oil-in-water emulsions (F/SPI and F/ SPI+SC+MTG) as described in Table 1.

Fig. 4. FT-IR spectra in the $1710\text{-}1590\text{ cm}^{-1}$ region of the frankfurters described in Table 1. A: F/PF; B: F/SPI; C: F/SPI+SC+MTG.

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