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 transforn 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, & Egelandsdal, 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-lactoalbumin, β-lactoglobulin) (Fang & Dalgleish, 1998; Lee, Lefévre, Subirade, & Paquin, 2009). Preliminary IR spectroscopy assays have been conducted at out 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, & Ruíz-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

69 more in line with nutritional recommendations. Of the procedures used to incorporate
70 oils as ingredients in the manufacture of meat derivatives, stabilization with oil-in-water
71 emulsions offers numerous advantages (Jiménez-Colmenero, 2007). Various procedures
72 have been reported to make pre-emulsions for addition to meat products (Jiménez
73 Colmenero, 2007, Cáceres et al., 2008). The most commonly employed are ones in
74 which protein systems like caseinate or soy protein isolate are used, although recently it
75 has been suggested that their activity could be assisted by means of other components
76 such as meat protein or microbial transglutaminase (Muguruma, Tsuruoka, Katayama,
77 Erwanto, Kawahara, & Yamauchi, 2003; Delgado-Pando, Cofrades, Ruiz-Capillas,
78 Solas, & Jiménez-Colmenero, 2010). In such cases, the use of these emulsions as animal
79 fat replacers in meat derivatives will influence the quality attributes of the product in
80 different ways. In this respect, studies dealing with the reformulation of healthy meat
81 products have focused mainly on aspects relating to changes in sensory characteristics
82 and technological properties, as these are the quality characteristics most appreciated by
83 consumers and they help to determine the commercial success of failure of any new
84 meat derivative. However, in order to develop better products of this kind we need to
85 gain a clearer understanding of the complex relationship between the structure of their
86 components and many of their quality-related (technological, sensory, etc.) properties.
87 Within this line of action, preliminary studies have been carried out on the
88 viability of IR spectroscopy to analyse the structural characteristics of healthier meat
89 derivatives formulated with olive oil-in-water emulsion stabilized with casein as a pork
90 backfat replacer (Carmona, Ruiz-Capillas, Jiménez-Colmenero, Pintado, & Herrero,
91 2012). These studies have highlighted some interesting possibilities that this technique
92 can offer in terms of changes in protein secondary structure and changes in lipid chain
93 order, or determination of lipid-protein interactions. These findings underline the

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

97 In this connection there is clearly a need to gain a better understanding of the role
98 played by different oil-in-water emulsion in both the technological properties and the
99 structural characteristics of the reformulated meat product. IR spectroscopy offers a
100 powerful tool with which to examine in depth the molecular changes that occur in both
101 lipids and proteins. The aim of this work was therefore to use FT-IR to elucidate lipid
102 and protein structural features of frankfurters formulated with an olive oil-in-water
103 emulsion stabilized with soy protein as a pork backfat replacer. A further aim was also
104 to establish possible connections between structural features and such technological
105 properties as texture of the final healthier meat product.
106

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 ºC. 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 ºC 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 ºC) (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 ºC 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 ºC) 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 CaF_2 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.

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

208 *2.6.2. Lipid structure analysis.* Spectral region 3000-2800 cm^{-1} was analyzed to
209 study lipid structure. In order to eliminate any spectral influence of water from the
210 samples in this spectral region (3000-2800 cm^{-1}), the spectral contribution of water was
211 appropriately subtracted from sample spectra using the 2125 cm^{-1} association band of
212 water as an internal intensity standard (Carmona et al., 2012). The water-free spectra of
213 the frankfurter were then subtracted, based on the elimination of the amide II band using
214 a subtraction factor so that the intensity maximum near 1545 cm^{-1} is not visible. The
215 half-bandwidths of the 2925 ($\nu_{as}CH_2$) and 2854 (ν_sCH_2) cm^{-1} bands were measured as
216 follows in the resulting difference spectra.

217 A straight line was drawn as a baseline tangentially between the absorbance
218 minima located on either side of the band in question (near 2990 and 2882 cm^{-1} for the
219 2925 cm^{-1} band, 2882 and 2810 cm^{-1} for the 2854 cm^{-1} band). The half-bandwidths for
220 each band are calculated by measuring the bandwidth at half height between the band
221 intensity maximum and the corresponding baseline.

222 *2.6.3. Protein structure analysis.* Spectral region 1700-1600 cm^{-1} was analyzed
223 to study protein structure. Cooked meat products like frankfurters are characterized by
224 high water content. Water exhibits strong absorption with a maximum located between
225 1650 and 1640 cm^{-1} (Herrero et al., 2010). This influences the protein structure
226 information which is determined through the amide I band (1700-1600 cm^{-1}) (Herrero et
227 al., 2010). Frankfurter samples were therefore deuterated to avoid this drawback in the
228 protein structure analysis. For deuteration, samples of about 1 mg weight were placed in
229 small glass tubes with an open side, which were placed in sealed glass containers 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 ºC. 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 D_2O 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 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. 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

255 the various secondary structure elements was estimated by dividing the integral
256 intensity of one amide I band component by the total intensity of all amide I band
257 components. On the basis of literature references, the bands appearing near 1682 cm^{-1}
258 are attributed to β-sheet structure, and those in the 1660-1650 cm^{-1} and 1640-1618 cm^{-1}
259 range are assigned to α-helix and β-sheet structures respectively (Barth, 2007). The
260 differences between samples of each secondary structure element are given in relative
261 percentage terms.
262
263 *2.7. Statistical analysis.*
264 Analysis of variance (ANOVA one-way) and Tukey´s multiple range test were
265 performed in order to evaluate the statistical significance ($P<0.05$) of the effect of the
266 different olive oil-in-water emulsions in the frankfurter formulation. The normal
267 distribution of samples was checked using the Shapiro–Wilks test. The Kruskal-Wallis
268 test was used to test samples that did not fit the normal distribution. Statistical analysis
269 was performed using Statgraphics Plus version 5.0.
270
271 **3. Results and discussion.**
272 *3.1. Proximate analysis*
273 Frankfurters presented some differences in proximate analysis which were
274 consistent with product formulation (Table 1). All sausages contained similar ($P>0.05$)
275 protein levels: 17.30±0.20, 17.83±0.15 and 18.05±0.29 % for control (F/PF), F/SPI and
276 F/SPI+SC+MTG respectively. Sausages were formulated with the same target muscle
277 protein, so that while the protein in the control frankfurter was from meat raw material
278 (meat and pork backfat), the other samples additionally contained the non-meat proteins
279 used to stabilized the olive oil emulsions (Table 1). There were no differences ($P>0.05$)

280 in the moisture content (range from 60.6±0.08 to 61.5±0.15 %) and ash percentages
281 (3.01±0.02 to 3.05±0.03 %) in the different frankfurter formulations. Fat content was
282 generally close to the target level and there were no differences (P>0.05) between
283 samples. The corresponding fat contents of F/PF, F/SPI and F/SPI+SC+MTG were
284 19.40±0.12, 18.08±0.19 and 18.16±0.40 % respectively. When pork backfat was totally
285 replaced by oil-in-water emulsion (containing 52% olive oil), samples had around 13 g
286 of olive oil per100 g of product.
287
288 *3.2. Texture Profile Analysis.*
289 Texture profile analysis parameters were affected by frankfurter formulation
290 (Table 2). Replacement of pork backfat with oil-in-water emulsion generally produced
291 an increase (P<0.05) of hardness, springiness, cohesiveness and chewiness. However,
292 frankfurters made with any of the different oil-in-water emulsions presented lower
293 (P<0.05) adhesiveness than those made with all pork fat (Table 2). Some authors have
294 observed increased firmness in cooked meat products prepared with pre-emulsified
295 vegetable and/or fish oil as animal fat replacers (Delgado-Pando et al., 2010; Jiménez-
296 Colmenero, Herrero, Pintado, Solas, Ruiz-Capillas, Carmona, 2010; Youssef, & Barbut,
297 2011; Shao, Zou,; Xu, Wu, Zhou, 2011; Carmona et al., 2012). Other authors (Cáceres,
298 E.; García, M. L.; Selgas, 2008) reported that, in general terms, sensory analysis has
299 shown a slight increase in hardness and work of shearing in mortadella (Spanish
300 bologna-type sausage) formulated with higher levels of pre-emulsified fish oil
301 irrespective of the fat content
302 In the present work, it is important to note that the differences in textural
303 properties between frankfurters (Table 2) are determined mainly by the characteristics
304 of each lipid phase and, probably, its role in the meat protein matrix. Results showed

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

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

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

335 The composition and characteristics of the lipid materials, in particular animal
336 fat or olive oil-in-water emulsion, used in the reformulation of frankfurters are
337 important factors determining the textural properties of final cooked meat products.
338 Various factors may contribute to the effect that substituting vegetable oils for animal
339 fat has on the texture of meat products. On the one hand there are those related with the
340 fat source characteristics and their distribution in the protein matrix (Hong, Lee, & Min,
341 2004; Martín, Ruiz, Kivikari, & Puolanne, 2008). In this connection, previous results in
342 meat batters suggested that different added lipids (soybean oil, pork fat or butter) and
343 thermal treatments induced different changes in textural properties (Shao et al., 2011).

344

345 *3.3. Infrared spectroscopy (FT-IR)*

346 *3.3.1. Lipid structure analysis.* Fig. 2 shows typical FT-IR spectra in the 3050-
347 2800 cm^{-1} region of the frankfurters reformulated (F/PF, F/SPI and F/SPI+SC+MTG).
348 These spectra show two predominant strong bands at 2922 and 2852 cm^{-1} which can be
349 assigned to asymmetric and symmetric stretching vibrations of the acyl CH_2 groups
350 respectively (Guillen & Cabo, 1997). Modifications of the half-bandwidth of these bands
351 can be generated by changes in lipid chain order/disorder resulting from protein-lipid
352 interactions (Fraile, Patrón-Gallardo, López-Rodríguez, & Carmona, 1999). Narrowing
353 of the spectral profile of these bands [2922 cm^{-1} ($\nu_{as}CH_2$) and 2852 cm^{-1} (ν_sCH_2)] is
354 generally attributed to increasing conformational order of lipid acyl chains (Fraile, et al.,

355 1999). The lipid concentration of the reformulated frankfurters from olive oil-in-water
356 emulsion or animal fat was approximately 13-14%, while the concentration from meat
357 endogenous lipids in these products was only 4-5%. Therefore, the lipid concentration
358 of these frankfurters comes mainly from the emulsion or animal fat used in their
359 reformulation. In this connection, the observed changes in lipid structure can be mainly
360 attributed to the incorporation of olive oil in water emulsion in F/SPI and
361 F/SPI+SC+MTG or animal in F/PF. We measured the corresponding half-bandwidths
362 (Fig. 3) to determine the differences between the lipid structures of the reformulated
363 frankfurters in terms of order/disorder of lipid acyl chains. The half-bandwidth values of
364 the 2922 cm^{-1} band were lower ($P<0.05$) when pork backfat was replaced by oil-in-
365 water emulsion (Fig. 3). These results indicate that inter- and intra-molecular lipid order
366 is greater in samples made with olive oil-in-water emulsion, probably due to weaker
367 lipid-protein interactions (Fraile et al., 1999; Herrero et al., 2011a: 2011b). Similar
368 findings on frankfurter lipid structure have been reported in a previous work using olive
369 oil-in-water emulsion stabilized with casein as a pork backfat replacer (Carmona et al.,
370 2012). The lipid concentration of the frankfurters formulated with olive oil-in-water
371 emulsion was 13.5% (data taken of preparation of olive oil-in-water emulsions and
372 Table 1) while the concentration of meat endogenous lipids was only 3.6%. Therefore,
373 the lipid concentration of these frankfurters comes mainly from the emulsion added. In
374 this connection, the observed changes in lipid structure can be attributed to the
375 incorporation of olive oil in water emulsion in the frankfurter formulation. For clarity
376 we included this statement in the manuscript

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

379 heating forming a gelled protein matrix (gel/emulsion system) (Gordon & Barbut,
380 1992).

381 Two components are present in the frankfurter reformulated with oil in-water-
382 emulsion as an animal fat replacer, namely oil-in-water emulsion and meat matrix,
383 where this emulsion has been included. In the preliminary formation of the oil-in-water
384 emulsion, the protein molecules diffuse to and are adsorbed at the oil/water interface
385 and form a continuous cohesive film (Das & Kinsella, 1990). The hydrophobic loops
386 orient in the apolar oil phase, while polar charged segments extend into the aqueous
387 phase, but most of the molecule occupies the interface, interacts with neighbouring
388 molecules, and imparts stability to the emulsion (Das & Kinsella, 1990). In a previous
389 work it was reported that the formation of oil in-water-emulsion stabilized with soy
390 protein involves lipid chain disorder or lipid-protein interactions (Herrero et al., 2011b).
391 These processes may limit the capacity of the lipid and protein components of olive oil-
392 in-water emulsion to participate in subsequent lipid-protein interaction in the product's
393 meat matrix. That is consistent with the results of the present work, which indicated
394 more inter- and intra-molecular lipid order (lower lipid-protein interaction) in
395 frankfurters containing emulsion. This would mean that physical entrapment of the lipid
396 phase (oil-in-water emulsion) plays a greater part in the stabilization process of these
397 reformulated products (F/SPI and F/SPI+SC+MTG) (gel contribution). By contrast, the
398 frankfurter formulated with animal fat (F/PF) showed the highest ($P<0.05$) lipid chain
399 disorder (more lipid-protein interaction) (Fig. 2), possibly because meat protein chains
400 can be inserted in major extent between the acyl chains of the animal fat. This would
401 involve more lipid- protein interactions, with the formation of a thin layer of proteins
402 around the fat globules (emulsion theory).
403

404 *3.3.2. Protein structure analysis.* The amide I band (1700 –1600 cm^{-1}) was used
405 to study the changes occurring in the protein when pork backfat was replaced with olive
406 oil-in-water emulsion in the formulation of the frankfurters (Fig. 4). This band includes
407 primarily the C=O stretching vibrations of the amide groups (coupled to in-plane
408 bending of the N-H and stretching of the C-N bonds) (Herrero et al., 2010).
409 Modification of spectral features (intensity and/or frequency) in the amide I
410 region indicates adjustments associated with secondary structures such as α-helix, β-
411 sheet or unordered. Infrared bands centred between approximately 1650 and 1658 cm^{-1}
412 may be considered characteristics of α-helical structure. Polypeptide backbones in β-
413 sheet conformation give rise to infrared bands between approximately 1620 and 1640
414 cm^{-1} (Herrero et al., 2010).
415 Comparison of spectral profiles of the amide I band reveals a shift of the
416 absorption maximum from 1632 to 1620 cm^{-1} in F/SPI+SC+MTG (Fig. 4). This spectral
417 change is indicative of changes in protein structure in terms of greater content of
418 aggregated intermolecular β-sheets (Kirschner et al., 2004; Böcker et al., 2006).
419 Estimates of secondary structure percentages were obtained by deconvolution of
420 the amide I band region (1700–1600 cm^{-1}). F/SPI+SC+MTG showed a significant
421 ($P<0.05$) increase (28%) of β-sheet structure content attributable to aggregated
422 intermolecular β-sheets in this frankfurter as described above. These results were
423 consistent with literature references reporting that replacement of animal fat with olive
424 oil-in-water emulsion is accompanied by an increase in β-sheet structure (Carmona et
425 al., 2012). Enrichment of aggregated intermolecular β-sheets as observed in the present
426 work could be related to the formation of a denser network providing more firmness in
427 F/SPI+SC+MTG (Table 2). Previous morphological studies have indicated that
428 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 derivates 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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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.

567 Table 1. Formulation (g) of frankfurters elaborated with pork backfat (F/PF) and

568 different olive oil-in-water emulsions (F/SPI and F/SPI+SC+MTG).

569

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

570 Additives added to all samples: 2.0 g/100 g NaCl; 0.30 g/100 g sodium
571 tripolyphosphate; 0.012 g/100 g sodium nitrite; 0.60 g/100 g flavouring and 0.05 g/100
572 g of liquid smoke.
573 F/PF: frankfurter formulated with pork backfat. F/SPI: frankfurter formulated with olive
574 oil-in-water emulsion stabilized with soy protein isolate (O/SPI). F/SPI+SC+MTG:
575 frankfurter formulated with olive oil-in-water emulsion stabilized with a mixture of soy
576 protein isolate, sodium caseinate and microbial transglutaminase (O/SPI+SC+MTG)
577

578

579 Table 2. Textural profile analysis (TPA) of frankfurters formulated with pork backfat

580 (F/PF) and different olive oil-in-water emulsion (F/SPI and F/SPI+SC+MTG).

581

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}

582 Samples formulated with the corresponding oil-in-water emulsion described in Table 1.
583 Means ± standard desviation. Different letters in the same column indicate significant
584 differences ($P<0.05$).
585
586

587

588 **FIGURE CAPTIONS**

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

592

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

595

596 Fig. 3. Half-bandwidth values of the 2922 ($\nu_{as}CH_2$) and 2852 (ν_sCH_2) cm^{-1} bands of
597 frankfurter formulated with pork backfat (F/PF) and olive oil-in-water emulsions (F/SPI
598 and F/ SPI+SC+MTG) as described in Table 1.

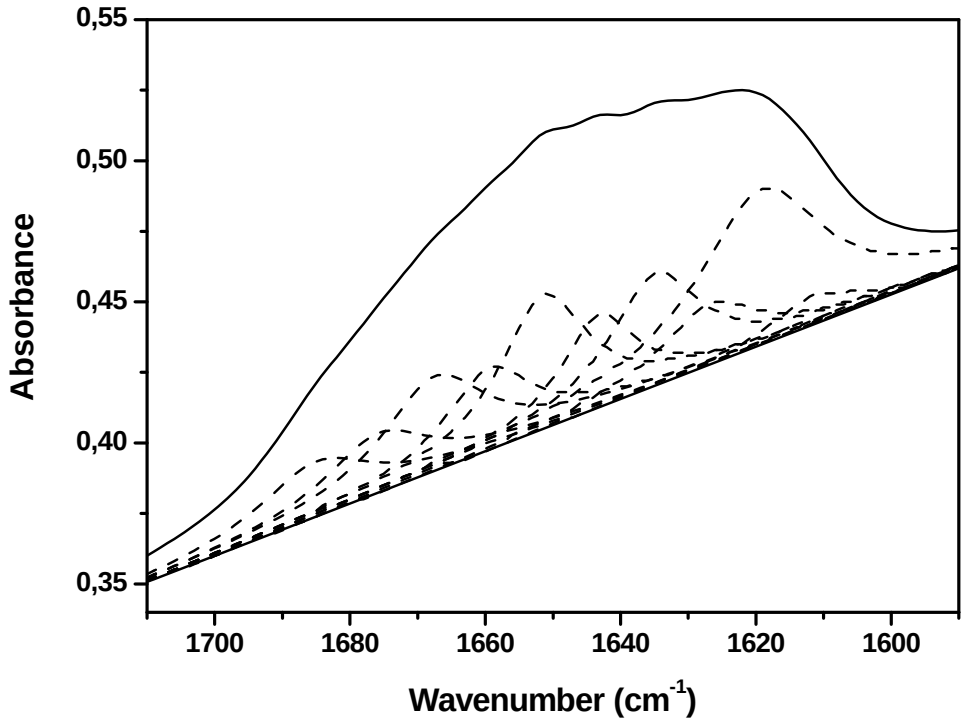
599

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

602

603 Fig. 1

604



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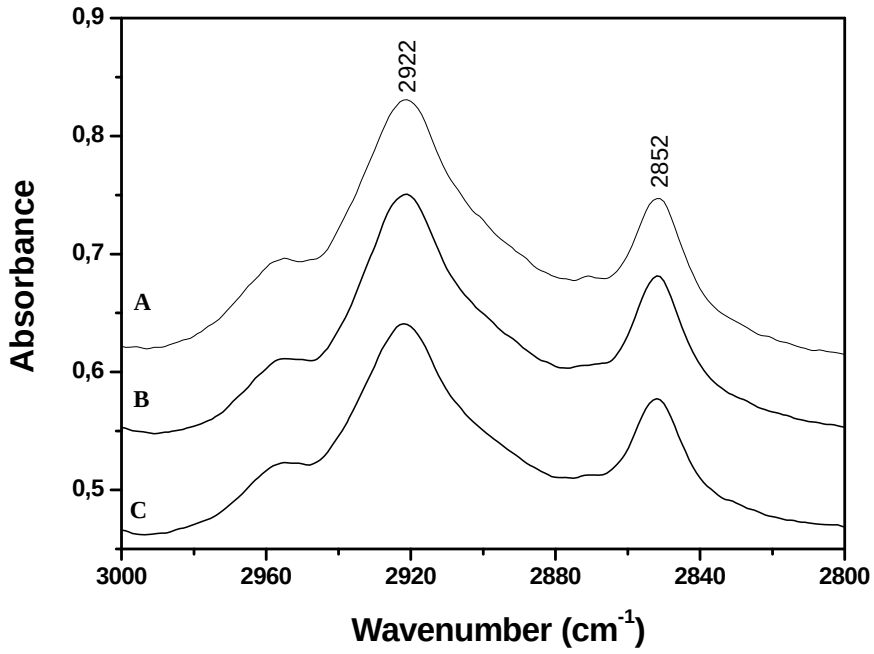
607 Fig. 2



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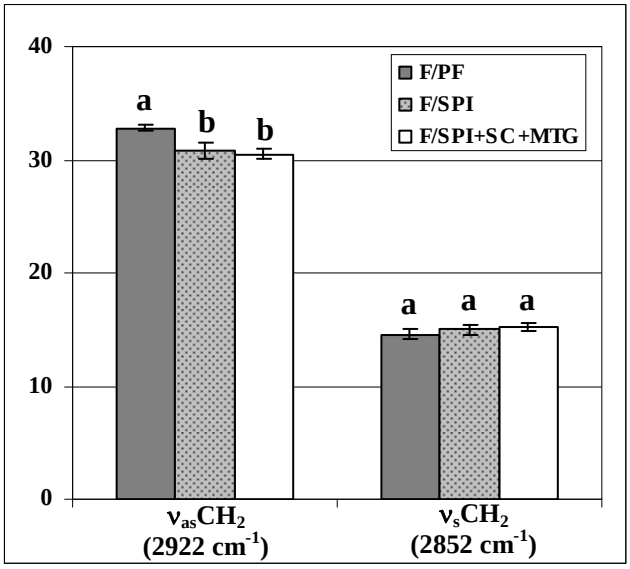
610 Fig. 3



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613 Fig. 4

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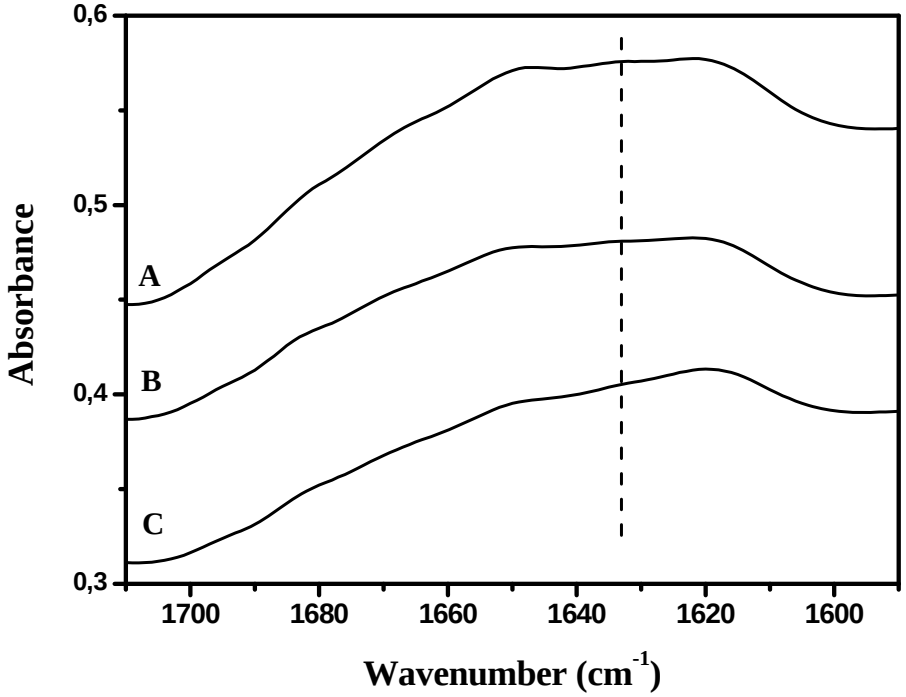

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