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Infrared spectroscopy used to determine effects of chia and olive oil incorporation strategies on lipid structure of reduced-fat frankfurters

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Abstract

This article reports an infrared spectroscopic study, using attenuated total reflectance (ATR-FTIR), on the structural characteristics of lipids in frankfurters as affected by different strategies to replace animal fat with chia and olive oil. Three incorporation strategies were considered: direct addition (FCO) and addition in a conventional emulsion (non-gelled) (FCE) or an emulsion gel using alginate as a gelling agent (FCEG). Reduced-fat (all-pork-fat) frankfurters (FP) were used as reference. Proximate composition and specific technological properties (pH, processing loss, texture) were also evaluated. FCE and FCEG frankfurters showed a shift to higher frequencies and the highest ($p < 0.05$) half-bandwidth in the $\nu_{as}CH_2$ and ν_sCH_2 bands. These spectroscopic results could be related to the fact that the lipid chain was more disorderly in these samples, presumably because there were more lipid interactions than in the reference frankfurter. These features of lipid structure correlated significantly with processing loss and textural behaviour.

Keywords: olive oil, chia, frankfurter, lipid structure, infrared spectroscopy, technological properties

Running title: Chia and olive oil incorporation strategies on frankfurters

1. Introduction

The idea of stabilising and structuring edible oils to promote solid–lipid functionality for use as an alternative to animal fat in the development of healthy lipid meat products has attracted considerable attention in recent years (Jimenez-Colmenero, Salcedo-Sandoval, Bou, Cofrades, Herrero, & Ruiz-Capillas, 2015). Of the procedures used one that offers numerous advantages is oil stabilisation in the form of structured emulsions (Jimenez-Colmenero et al., 2015). Conventional emulsions consist of at least two immiscible phases (usually oil and water), with one phase dispersed in the other as tiny droplets. They are usually classified according to the arrangement of the two immiscible liquids as either oil-in-water (O/W) or water in-oil (W/O) systems, but they are not normally able to provide a solid-like texture. However, one of the most promising options is emulsion gels. An emulsion gel is defined as an emulsion with a gel-like network structure and solid-like mechanical properties (Dickinson, 2012, 2013). Emulsion gel formulation usually involves producing a protein-stabilised emulsion and incorporating a hydrocolloid stabiliser or other ingredients (protein, polysaccharides, surfactant, etc.) once the emulsion is formed, to produce an emulsion gel either by aggregation of the emulsion droplets or by gelling of the continuous phase (Dickinson, 2012, 2013).

The use of structured emulsions, particularly O/W emulsion gels, as animal fat replacers may be particularly suited to the design and development of healthier meat products, not only because of their technological characteristics but also because both lipid sources and emulsifying compounds with healthy properties can be used in their preparation. In this regard olive oil, already widely accepted and known for its pleasant flavour, contains antioxidants which also confer health benefits (López-Miranda, Pérez-Martinez, & Pérez-Jiménez, 2006). Additionally, compounds of plant origin such as chia *(Salvia hispanica* L.) could offer promising possibilities as ingredients, given their useful technological properties, such as sufficient emulsifying activity to provide high emulsion stability (Olivos-Lugo, Valdivia-Lopez, & Tecante, 2010; Pintado, Ruiz-Capillas, Jimenez-Colmenero, Carmona, & Herrero, 2015). This ingredient has also been investigated and recommended for the major positive effect that it has on human health, particularly through its lipids, mainly high α-linolenic fatty acid and dietary fibre content (Ayerza & Coates, 2004, 2011; Olivos-Lugo et al., 2010).

The strategies used to incorporate those functional and/or technological ingredients as animal fat replacers in the development of healthier-lipid meat products can affect not only the composition but also the technological and organoleptic properties of such reformulated meat products (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2010; Jimenez-Colmenero, Herrero, Pintado, Solas, & Ruiz-Capillas, 2010; Pintado, Herrero, Jimenez-Colmenero, & Ruiz-Capillas, 2016a; Pintado, Herrero, Ruiz-Capillas, Triki, Carmona, & Jimenez-Colmenero, 2016b; Poyato, Ansorena, Berasategi, Navarro-Blasco, & Astiasaran, 2014). To advance in the development of this kind of food, it would also be desirable to examine the changes occurring in these quality characteristics in the final reformulated product and the role that lipid structure plays in these properties.

The goal of the present study was, then, using infrared spectroscopy (FTIR), to determine how the lipid structure was influenced by the presence and the manner of addition of healthy ingredients, chia flour and olive oil, to replace animal fat in reduced-fat frankfurters. For this purpose both ingredients were added either directly, in the form of an O/W conventional emulsion or as an emulsion gel, to establish the relationships between lipid structure and specific quality-related technological properties (processing loss and texture) of final products. Reduced-fat frankfurters (all-pork-fat) were used as reference. The advantages of attenuated total reflectance (ATR)-FTIR spectroscopy were expected to provide insights into the behaviour of the lipid material at a molecular level (Chalmers & Griffiths, 2010; Wilson & Tapp, 1999).

2. Material and Methods

2.1. Elaboration of chia emulsions

Two different chia oil-in-water (O/W) emulsions were prepared for use as animal fat replacers (Pintado et al., 2015) (Fig. 1): 1) a conventional chia oil-in-water (O/W) (non-gelled) emulsion labelled CE, prepared with olive oil (16.80%) (Carbonell Virgen Extra, SOS Cuétara S.A., Madrid Spain), water (57.36%) and chia flour (*Salvia hispanica* L.) (25.84%) (Primaria Premium Raw Materials, S. L. Valencia, Spain]; and 2) a chia O/W emulsion gel labelled CEG made with olive oil (16.80%), water (55.36%), chia flour (25.84%) and a cold gelling agent based on alginate (2%) [sodium alginate (0.73%) (Tradissimo, TRADES S.A., Barcelona, Spain), $CaSO_4$ (0.73%) and pyrophosphate (0.54%) (Panreac Química, S.A. Madrid, Spain)]. Chia contained 22% protein and 31% fat, according to information provided by the supplier.

Briefly, these emulsions were prepared (in duplicate) as follows: first chia flour was mixed with water or, in the case of CEG, with an aqueous solution of gelling agent, for 45 s at high speed, using a homogeniser (Thermomix™ 31; VorwerkEspaña M.S.L., S.C, Madrid, Spain). Then the final mixture was mixed again (3 min, approx. 5600 rpm) while gradually adding olive oil (Pintado, et al., 2015). Finally, each type of emulsion was placed in a metal container under pressure to compact it and prevent air bubbles from forming, and then stored in a chilled room at 2 ºC for 24 h.

2.2. Preparation of frankfurters

Sufficient (35 kg) fresh post-rigor pork (mixture of *biceps femoris*, *semimembranosus*, *semitendinosus*, *gracilis and adductor* M) (22.1% ± 0.4 protein, 5.08% ± 0.6 fat) and pork backfat (5 kg) (7.5% ± 0.7 protein, 86.7% ± 1.9 fat), both from different animals, were obtained from a local market on different days.

Four different reduced-fat (about 13%) frankfurter types were prepared (Table 1) and analysed (Fig. 1). One was formulated as control with all pork fat (FP), and the other three reformulated by totally replacing pork backfat with an identical amount of chia flour (10%) and olive oil (6.5%) but incorporated following different procedures: direct addition (FCO); in the form of a conventional O/W chia (non-gelled) emulsion (FCE); or as an O/W chia emulsion gel (FCEG) (Table 1) (Fig. 1). FCE and FCEG comprise 38.7% of emulsion which contain 16.8% of olive oil and 25.84% of chia flour.

Frankfurters were prepared according to Jimenez-Colmenero et al. (2010). Briefly, the ingredients were thoroughly mixed in a Stephan Universal Machine UM5 (Stephan u. Söhne GmbH and Co., Hameln, Germany) at 2 ºC and the resulting meat batter was stuffed into 20 mm diameter Nojax cellulose casings (Viscase S.A., Bagnold Cedex, France). Samples were hand linked and heat processed in an Eller smokehouse (model Unimatic 1000, Micro 40; Eller, Merano, Italy). Frankfurters were then cooled (at room temperature), removed from their casings, vacuum packed in plastic bags (Cryovac® BB3050, Boi de Llobregat, Spain) and stored at 2 °C (±1 °C) until analysis (Fig. 1). The entire meat system processing procedure was replicated twice on two different days.

2.3. Proximate Analysis

Moisture and ash contents were determined in triplicate (AOAC, 2005). Protein content was measured in triplicate using an FP-2000 nitrogen analyser (Leco Corporation, St Joseph, MI). Fat content was evaluated in triplicate according to Bligh and Dyer (1959).

2.4. Processing loss and pH

Processing loss was calculated in ten frankfurters, as the weight loss (expressed as percentage of initial sample weight) occurring after heat processing and chilling overnight at 2 ºC.

pH values were measured in quadruplicate using an 827 Metrohm pH-meter (Metrohm AG, Herisau, Switzerland) at room temperature on homogenates (ratio of 1:10 *w/v* of sample/distilled water).

2.5. Textural properties

Textural properties were analysed (six times) by texture profile analysis (TPA) performed in a TA-XT.plus texture analyser (Texture Technologies Corp., Scarsdale, NY) as described by Bourne (1978). Frankfurter sections (height = 20 mm) were axially compressed to 40% of their original height. Force–time deformation curves were obtained with a 5-kg load cell, applied at a crosshead speed of 0.8 mm/s. Attributes calculated were: hardness (Hd) (N), cohesiveness (Ch) (dimensionless), springiness (Sp) (mm) and chewiness (Cw) (N*mm).

2.6. Attenuated total reflectance (ATR)- FTIR spectroscopic analysis

The infrared spectra of each type of sample were recorded using the Perkin-Elmer Spectrum™ 400 spectrometer (Perkin Elmer Inc., Madrid, Spain) in mid-IR mode, equipped with an attenuated total reflectance (ATR) sampling device containing diamond/ZnSe crystal. For spectroscopic analysis 25 mg of each sample (with no previous sample preparation) were placed on the surface of the ATR crystal and slightly pressed with a flat-tipped plunger. Spectra were scanned in the wave number range of 4000–650 cm^{-1}, at a scan speed of 0.20 cm/s, and 8 accumulations at a resolution of 4 cm^{-1}. Measurements were performed on three different pieces for each type of sample. Three different portions were recorded for each sample and spectra were summed giving a final spectrum of 24 scans. A total of three sum spectra (72 accumulations) for each type of sample was analysed. Spectra were acquired with Spectrum version 6.3.2 software and spectral data were treated with Grams/AI version 9.1 (Thermo Electron Corporation, Waltham, MA,) software.

2.7. Statistical analysis

The entire trial was replicated. One-way analysis of variance (ANOVA) was performed to evaluate the statistical significance ($p < 0.05$) of the effect of frankfurter formulation, using the SPSS Statistics general linear model (GLM) procedure (v.22, IBM SPSS Inc.; Chicago, IL). Formulation was assigned as a fixed effect and replication as a random effect. Least squares differences were used for comparison of mean values among formulations and Tukey's HSD test to identify significant differences ($p < 0.05$) between formulations. Pearson product moment correlations (r) were performed to determine statistically significant relationships between data obtained by processing loss, TPA and Raman spectroscopy analysis focused on pairs of variables. P-values were used to test the statistical significance of the estimated correlations.

3. Results and Discussion

3.1. Proximate Analysis

Proximate analyses of frankfurters were affected ($p < 0.05$) by formulation (Table 2). Moisture contents ranged from 64 to 69%, with higher ($p < 0.05$) values occurring in samples with all pork fat (FP), followed by samples with chia and olive oil added directly (FCO) as per formulation (Table 1). All the frankfurters reformulated with chia flour (FCO, FCE and FCEG) had higher ($p < 0.05$) protein contents (Table 2), irrespective of the incorporation strategy used; about 2.2% of the protein in these samples came from chia. Chia protein has a high-quality amino acid profile and contains no gluten (Ayerza & Coates, 2011). Fat content was close to the target level, between 12.5% and 13.2%, with no significant differences between samples (Table 2). The lipid content in FP samples came from lean pork meat and pork backfat (all pork fat), whereas in the reformulated frankfurters (FCO, FCE and FCEG) the lipid content came from meat ingredients, chia and olive oil (Table 1). Hence, given the nature and composition of their lipid components, the chia and olive oil could improve the fatty acid profiles of frankfurter by supplying a considerable input of linolenic acid and MUFAs (Ayerza & Coates, 2011; Pintado et al., 2016a,b). The proportion of ash was also affected ($p < 0.05$) by formulation, with the highest ($p < 0.05$) values occurring in FCEG samples, possibly due to the added salts used for alginate gelification (Pintado et al., 2015).

3.2. Processing loss and pH

Processing loss of frankfurter ranged between 12_17%, a level that may be considered normal in products of this kind, including those reformulated with animal fat replacers, such as structured emulsions, oil bulking agents, etc. (Delgado-Pando et al., 2010; Herrero, Ruiz-Capillas, Jiménez-Colmenero, & Carmona, 2014; Pintado et al., 2016a). Frankfurters with all animal fat (FP) registered the greatest processing loss, probably due to their lower protein content (as compared to the other samples), resulting in a meat matrix with poorer water-binding properties (Choi et al., 2014; Jimenez-Colmenero et al., 2010). Comparison of samples reformulated with chia and olive oil (FCO, FCE and FCEG) showed that processing loss depended on the way in which these ingredients had been incorporated. Samples made with chia and oil in an O/W emulsion gel (FCEG) registered the lowest ($p < 0.05$) values, while samples prepared with chia and olive oil added directly (FCO) or in a non-gelled emulsion (FCE) registered the highest ($p < 0.05$) values. This may be because in the frankfurters reformulated with chia and oil in an emulsion gel (FCEG) part of the water and fat in the emulsion may could be more strongly embedded and bound due to the gelling agent.

It has been shown that incorporation of gels as animal fat replacers in low-fat cooked meat products improves emulsion stability, in terms of its water and fat binding properties (Fernández-Martín, López-López, Cofrades, & Colmenero, 2009; Ruiz-Capillas, Carmona, Jiménez-Colmenero, & Herrero, 2013). Emulsification processes with vegetable oil replacing back fat have generally resulted in cooked meat products with improved water and fat binding properties (Jimenez-Colmenero et al., 2010; Youssef, Barbut, & Smith, 2011; Zhuang et al., 2016). By using oil emulsion technology the oils can be stabilised or immobilised in a protein matrix, which reduces the chances of bulk oil physically separating from the structure of the meat product, so that it remains stable during processing and storage (Jimenez-Colmenero, 2007).

The pH values were similar ($p > 0.05$) for all samples and were within the normal parameters for this kind of product, between 6.3 and 6.4 (Bloukas & Paneras, 1993; Jiménez-Colmenero, Cofrades, Herrero, Fernández-Martín, Rodríguez-Salas, & Ruiz-Capillas, 2012; Jimenez-Colmenero et al., 2010; Pintado et al., 2016a; Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, 2013).

3.3. Textural properties

Fig. 2 shows that TPA parameters of frankfurters were affected ($p < 0.05$) by the formulation (including chia and olive oil incorporation strategy). All reformulated frankfurters registered higher ($p < 0.05$) hardness than control samples (FP) (Fig. 2). It has been established that the more protein a meat product contains, as in the case of FP (Table 2), the smaller the possibility of the interactions that build gel/emulsion structures (as in frankfurter), so that the matrices have poorer binding properties and a softer texture (Fig. 2) (Cavestany, Colmenero, Solas, & Carballo, 1994; Claus, Hunt, &

Kastner, 1989). It has also been reported that oils achieve a better distribution than animal fat in meat emulsion matrices, thus producing firmer sausages, due to improved association with the protein (Delgado-Pando et al., 2010). Additionally, the presence of chia flour could influence the textural behaviour of frankfurter because of the technological properties of chia flour, which supply sufficient emulsifying activity to provide high emulsion stability as well as useful gelling properties (Ayerza & Coates, 2011; Coorey, Tjoe, & Jayasena, 2014; Olivos-Lugo et al., 2010). Particularly when comparing frankfurters reformulated with chia and oil incorporated by means of different strategies (FCO, FCE and FCEG), cohesiveness and springiness were similar ($p > 0.05$) in all cases but lower ($p < 0.05$) than in the sample with all pork fat (FP) (Fig. 2). However, hardness and chewiness were higher ($p < 0.05$) in the samples reformulated with chia and olive oil in a conventional O/W emulsion (FCE) or an emulsion gel (FCEG) (Fig. 2). Similarly, previous studies have shown that frankfurters made with different O/W emulsions possessed greater hardness, cohesiveness and chewiness than a control formulated with all pork fat, and that this increase was more pronounced when a gelling agent was included in the emulsion (Jimenez-Colmenero et al., 2010; Pintado et al., 2016b; Zhuang et al., 2016). Other authors have concluded that gelled emulsions as animal fat replacers maintain the hardness of normal fat cooked meat product more efficiently (Poyato et al., 2014).

On the other hand, the importance of the sensory evaluation in terms of texture and other sensory parameters is relevant to understand better the acceptance of these products. In this context, previous findings in similar reformulated meat products with chia emulsion as fat replacer indicated that although differences were detected in the sensory attributes of frankfurters reformulated with chia, these products were judged acceptable by panellists (Pintado et al., 2016a, b).

3.4. Attenuated total reflectance (ATR)-FTIR spectroscopic analysis

Spectroscopic techniques, including ATR-FTIR, can be used in muscle foods both to determine structural changes in muscle food components during processing and storage and as a tool for quality assessment. However, these methodologies require a highly homogeneous matrix, and this limits their potential application to real meat products. Thus, finely-comminuted meat products like the ones considered in this study (frankfurters, a widely-accepted product in certain population groups) are exceptionally well suited to test the application of these methodologies in real complex systems. These are matrices with the very high level of structural disintegration essential to achieve representative findings (using ATR-FTIR) on the molecular structural characteristics of lipids and relate these to the technological properties of products. As these are real products, the results may be more directly applicable without the limitations imposed by the need to extrapolate when the study is conducted on model systems. A very common problem when using these spectroscopic techniques in food studies are changes at a molecular level.

The spectral region 3000–2800 cm^{-1} was analysed to study the influence of the various chia and olive oil incorporation strategies on lipid structure (Fig. 3). To rule out any spectral influence of water in the frankfurter formulated with pork fat (FP) in the 3000–2800 cm^{-1} region, the spectral contribution of water was duly subtracted from sample spectra using the 2125 cm^{-1} association band of water as an internal intensity standard, as it has been reported to be insensitive to the micro-environment (Lavialle, Adams, & Levin, 1982; Vincent, Steer, & Levin, 1984). In all frankfurters formulated with chia and olive oil (FCO, FCE and FCEG) to avoid any spectral influence of water and the rest of ingredients (lipids, fibre, etc.) the corresponding aqueous solution spectrum was appropriately subtracted accordingly, again the 2125 cm^{-1} association band of water as an internal intensity standard (Lavialle et al., 1982; Vincent et al., 1984; Herrero et al., 2011; Herrero et al., 2012). In addition, to avoid any spectral influence of proteins in this spectral region (3000–2800 cm^{-1}), the resulting spectra of the frankfurters were then subtracted using a subtraction factor to eliminate the amide II band, so that the intensity maximum near 1545cm^{-1} was not visible.

The typical resulting infrared spectra in the 3000–2800 cm^{-1} are shown in Fig. 3, where two strong bands from characteristic common lipid functional groups can be seem, at about 2919 and 2851 cm^{-1} for FP. These bands are the result, respectively, of the asymmetric and symmetric stretching vibrations of the acyl CH_2 groups (Guillen & Cabo, 1997; Herrero, Carmona, Pintado, Jimenez-Colmenero, & Ruiz-Capillas, 2011). The alterations of these infrared bands in terms of frequency and broadening are generally attributed to changes in the conformational order of the lipid acyl chains and to their dynamics (Fraile, Patron-Gallardo, Lopez-Rodriguez, & Carmona, 1999; Herrero et al., 2011). The interactions of lipids with other biomolecules such as proteins normally generate spectral changes of the methylene νCH modes of lipid chains, more pronounced in the asymmetric bands ($\nu_{as}CH_2$) than in symmetric ($\nu_{s}CH_2$) bands, here due to the breaking of Fermi resonance between the νCH fundamental (at about 2900 cm^{-1}) and binary combinations of CH_2 bending modes (at about 1460 cm^{-1}) (Kodati, Eljastimi, & Lafleur, 1994). The intensity maxima of these bands ($\nu_{as}CH_2$ and $\nu_{s}CH_2$) shifted to higher frequencies in frankfurters reformulated with all animal fat (FP) and in those reformulated with chia and olive oil added directly (FCO), chia O/W non-gelled emulsion (FCE) and emulsion gel (FCEG) (Fig. 3). These increases of frequency could be the result of increased conformational disorder in the lipid acyl chains (Fraile et al., 1999). It is thus possible to follow the transition from the ordered lipid phase in the samples formulated with animal fat (FP) to a more disordered lipid phase in frankfurters reformulated with chia and olive oil added directly (FCO), in a conventional emulsion (FCE) or in an emulsion gel (FCEG). This effect was more pronounced in the last two (Fig. 3). Previous findings (Pintado et al., 2015, 2016b) suggest that the reason for this frequency upshift could be that there were more lipid interactions when a conventional emulsion or emulsion gel was used in replacing animal fat.

In order to extract more precise and quantitative information on lipid structure as a function of chia and olive oil incorporation strategy, half-bandwidth values of $\nu_{as}CH_2$ and ν_sCH_2 were determined in each type of sample. These half-bandwidths were measured as follows. A straight line was drawn as a baseline tangentially between the absorbance minima located on either side of the band in question. The half-bandwidths for each band are calculated by measuring the bandwidth at half height between the band intensity maximum and the corresponding baseline. Results of half-bandwidths of $\nu_{as}CH_2$ and ν_sCH_2 bands are shown in Table 3. The half-bandwidths of these bands increased significantly in descending order from FP to FCO < FCE or FCEG (Table 3), reflecting a converse increase in the conformational disorder of lipid acyl chains. These results confirm the frequency analyses, in the sense that inter- and intramolecular lipid disorder was greater in samples to which the chia and oil were added directly, and even more so in samples where they were added in the form of a conventional emulsion or an emulsion gel (FCE or FCEG) which produced more lipid interactions in these products. On that assumption, the increasing lipid chain disorder observed from FP < FCO < FCE or FCEG could be due to the fact that in reformulated samples containing chia and olive oil more meat protein chains can be inserted between the acyl chains of the oil (in liquid, non-gelled emulsion or emulsion gel form) than in frankfurters containing pork fat. This would imply more lipid–protein interactions in the reformulated products (FCO, FCE or FCEG) (Table 3). Similar behaviour was observed when comparing frankfurters formulated with animal fat and reformulated with an emulsion gel or an oil bulking agent containing alginate as animal fat replacer (Herrero et al., 2014; Pintado et al., 2016b). On the other hand, there was less inter- and intra-molecular lipid disorder in frankfurters reformulated with O/W emulsion containing caseinate or soy protein without or with transglutaminase (MTG) as a fat replacer, which suggests that there were less lipid–protein interactions in these meat derivatives (Herrero et al., 2011, 2012). These differences in lipid structure features possibly arise from differences in proteins (type and/or concentration), lipid content and/or gelling agent used to make the emulsion. In frankfurter manufacture there are two states: an O/W emulsion as animal fat replacer, and a meat matrix in which this emulsion is incorporated. It seems that the protein–lipid interactions in O/W emulsions containing caseinate, SPI and/or MTG permit their stabilisation but may limit their capacity (less lipid chain disorder) for subsequent lipid–protein interaction in the product's meat matrix (Herrero et al., 2011, 2012). However, in the chia emulsion non-gelled (FCE) or gelled with alginate (FCEG), considered in this study, lipid interactions could have played a significant role both in stabilisation of the emulsion and in final formation of the meat matrix, by promoting lipid–protein interactions (greater lipid chain disorder) between the components of the olive O/W emulsion and the meat protein.

3.5. Relationship between lipid structure characteristics and processing loss or textural properties

The changes in lipid structure (in terms of half-bandwidth values, Table 3) as a function of differences in lipid composition and chia and olive oil incorporation strategies (Table 1) were accompanied by differences in technological properties such as processing loss and texture of frankfurters. For instance, there was a significant positive correlation between the half-bandwidth values of the $\nu_{as}CH_2$ (r = 0.82; p <

0.01) and $\nu_s CH_2$ (r = 0.80; p < 0.01) bands and processing loss values. Additionally, there was a significant correlation between hardness (r = 0.93; p < 0.00001), cohesiveness (r = –0.85; p < 0.001), springiness (r = –0.69; p < 0.05), chewiness (r = 0.72; p < 0.05) and half-bandwidth of $\nu_{as} CH_2$. Similarly, there was a significant correlation between hardness (r = 0.80; p < 0.005), cohesiveness (r = –0.78; p < 0.005), springiness (r = –0.61; p < 0.05), chewiness (r = 0.60; p < 0.05) and half-bandwidth of $\nu_s CH_2$.

These correlations suggest that the observed changes in lipid structure, in terms of order/disordering in oil acyl chains or lipid interaction, resulting from reformulation and different animal fat replacement strategies could be decisive for processing loss as a consequence of differences in the water and fat binding properties and the textural behaviour of the final meat product. This is consistent with reports in the literature that substitution of pork backfat with plant oils in a pre-emulsification step resulted in secondary protein structural changes, in terms of augmented of β-sheet structure, accompanied by better water binding properties and stronger texture (Carmona, Ruiz-Capillas, Jimenez-Colmenero, Pintado, & Herrero, 2011; Herrero et al., 2012; Xiong, Han, Kang, Zhao, Xu, & Zhu, 2016).

4. Conclusions

Infrared spectroscopy provided useful information on how features of lipid structure were affected by reformulation based on different lipid contents and strategies for incorporation of chia and olive oil, which included addition direct, in a conventional O/W emulsion (non-gelled) or in an emulsion gel, as animal fat replacers. It is important to note that when chia O/W non-gelled emulsion or emulsion gel was used as replacers, it caused increased lipid acyl chain disorder, involving more lipid–protein interactions. It seems that lipid interactions could play a significant role both in stabilising the emulsion and in final formation of the meat product matrix. These lipid structure characteristics affect some quality-related technological properties of the final reformulated meat product, which are very important for consumer acceptance, such as processing loss and texture. This information can be helpful in improving conditions for the development and reformulation of meat products, where animal fat is replaced, so as to achieve a product with a healthier lipid content.

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Figure captions

Fig. 1. Schematic illustration of the experiment.

Fig. 2. Texture profile analysis (TPA) parameters [hardness (Hd), cohesiveness (Ch), springiness (Sp) and chewiness (Cw)] of frankfurters described in Table 1. Different letters (a, b, c) indicate significant ($p < 0.05$) differences in the same parameters for the different frankfurters. For sample denominations see Table 1.

Fig. 3. ATR-FTIR spectral region (3000–2800 cm^{-1}) of frankfurters. For sample denominations see Table 1.

Fig. 1.

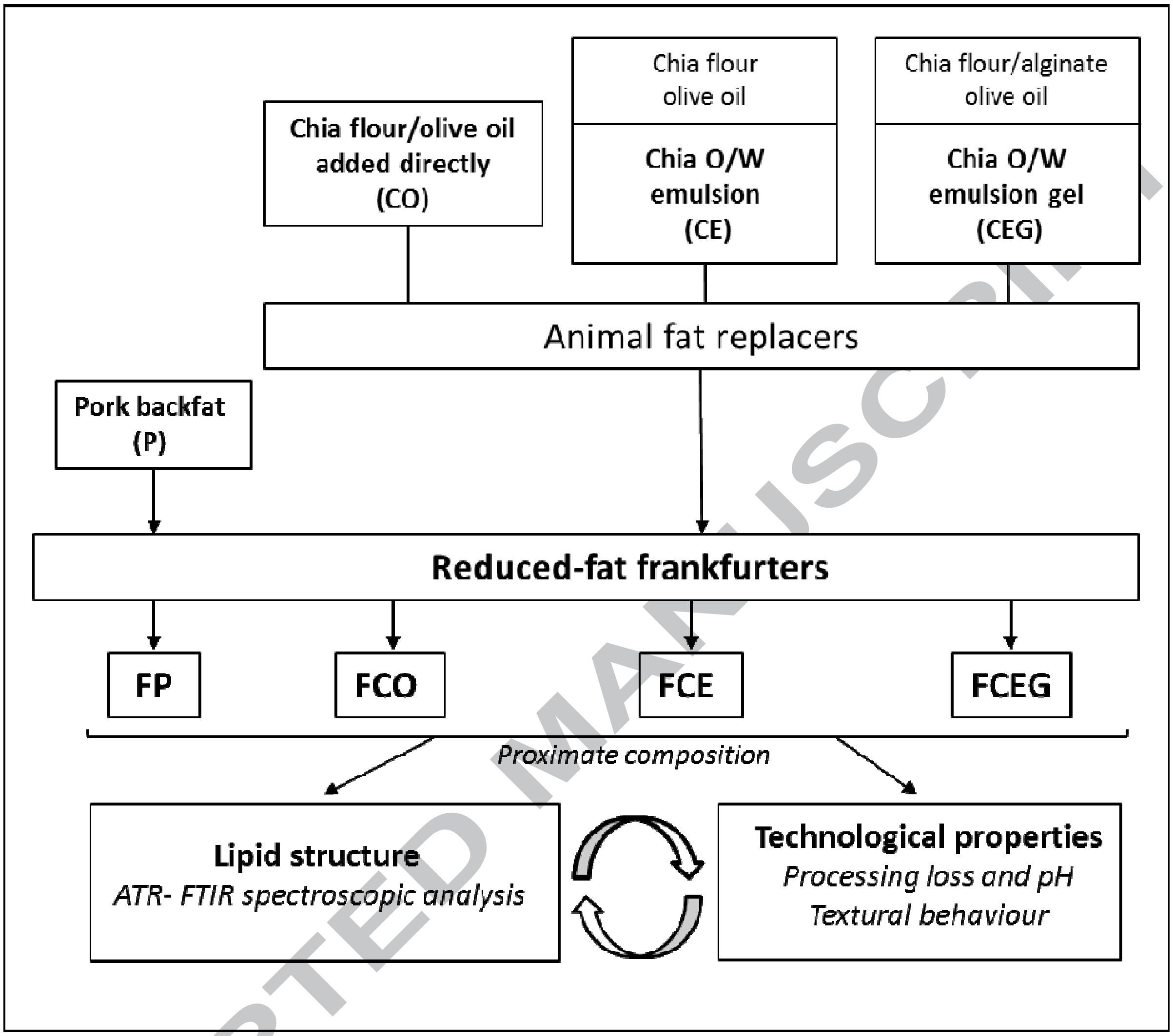

Chia flour/olive oil added directly (CO)
Chia flour olive oil
Chia O/W emulsion (CE)
Chia flour/alginate olive oil
Chia O/W emulsion gel (CEG)
Animal fat replacers
Pork backfat (P)
Reduced-fat frankfurters
FP
FCO
FCE
FCEG
Proximate composition
Lipid structure
ATR- FTIR spectroscopic analysis
Technological properties
Processing loss and pH
Textural behaviour

Fig. 2

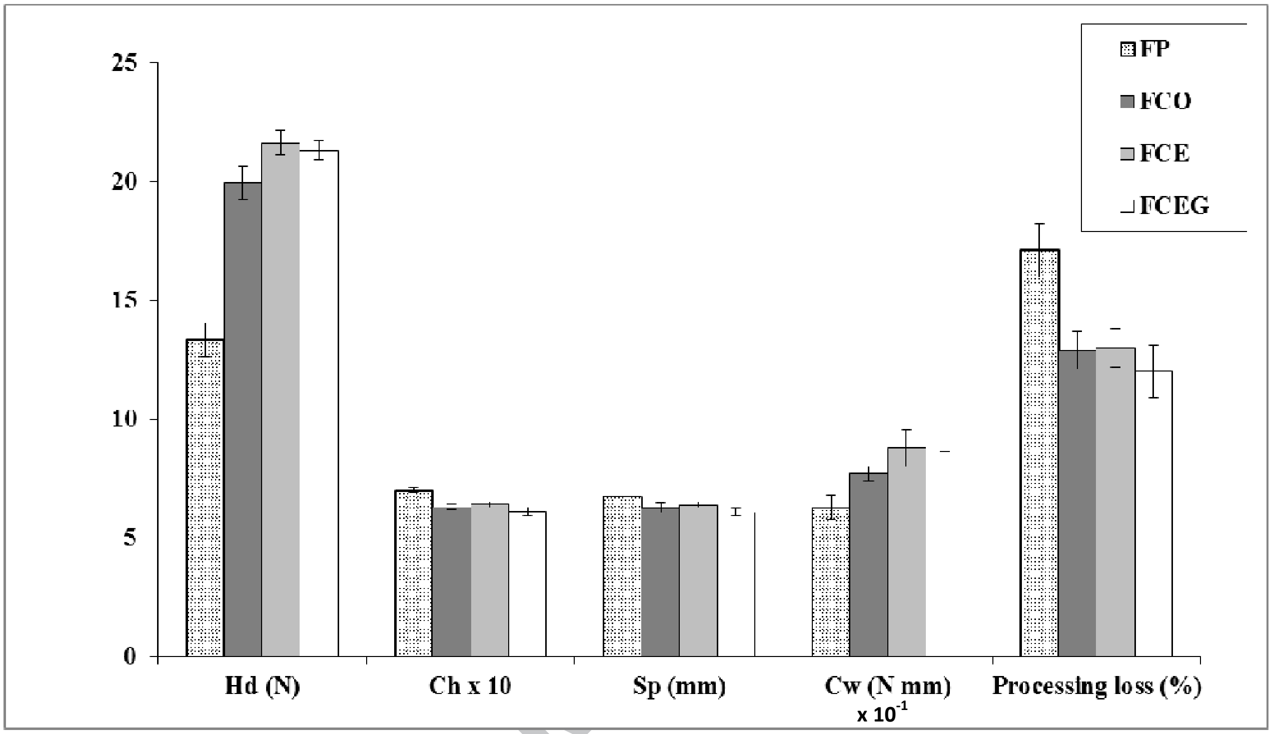
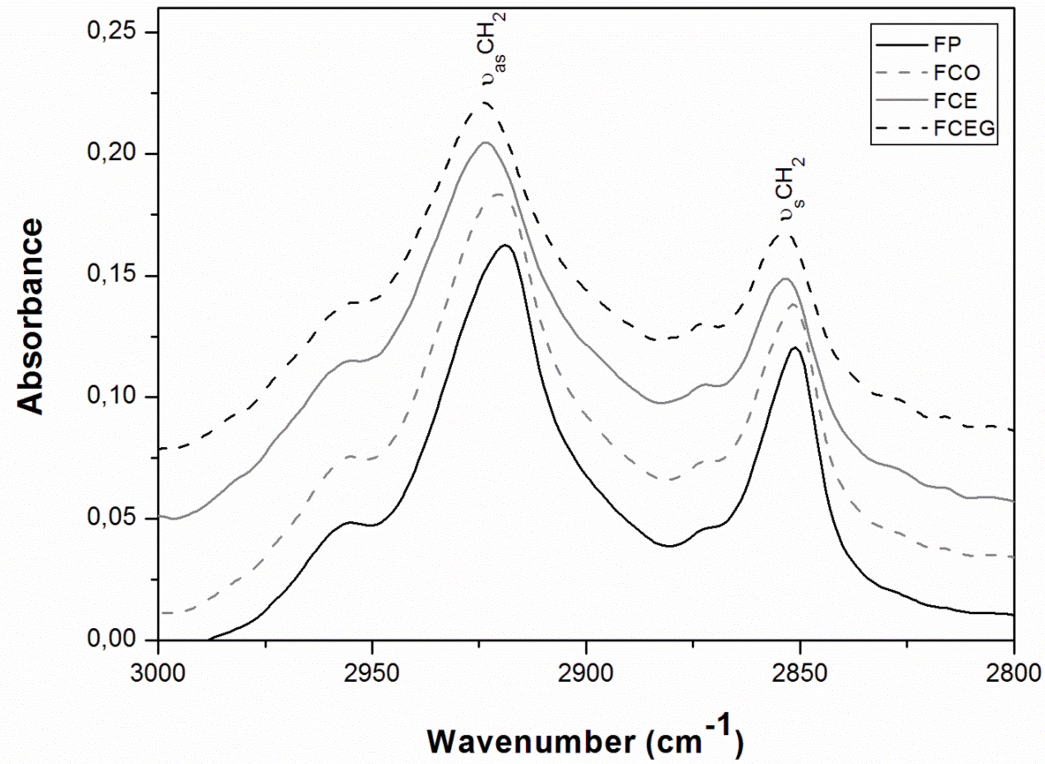

25
20
15
10
5
0
FP
FCO
FCE
FCEG
Hd (N)
Ch x 10
Sp (mm)
Cw (N mm) x 10-1
Processing loss (%)

Fig. 3.



FP
FCO
FCE
FCEG
υasCH2
υsCH2
Absorbance
0,00
0,05
0,10
0,15
0,20
0,25
3000
2950
2900
2850
2800
Wavenumber (cm-1)

Table 1. Formulation (%) of frankfurters.

Samples*	**Meat**	**Pork back fat**	**Chia flour**	**Olive oil**	**CE**	**CEG**	**Water**
FP	55.0	11.0		-----	-----	-----	31.7
FCO	55.0	-----	10	6.5	-----	-----	26.2
FCE	55.0	-----		-----	38.7	-----	4.0
FCEG	55.0	-----		-----	-----	38.7	4.0

Additives added to all the samples per 100 g of product: 1.5 g NaCl; 0.3 g sodium tripolyphosphate; 0.5 g flavouring and 0.012 g sodium nitrite.

*Reduced fat frankfurter formulated with pork backfat (control sample, FP) and reformulated by totally replacing of pork backfat with an identical amount of chia flour and olive oil added: directly (FCO) or incorporated in a non-gelled emulsion CE (labelled FCE), or in a gelled emulsion CEG (labelled FCEG).

Table 2. Proximate analysis (%) of frankfurters.

Samples*	Proximate analysis			
	Moisture	Protein	Fat	Ash
FP	69.0 ± 0.2 [c]	14.0 ± 0.2 [a]	12.5 ± 0.8 [a]	2.70 ± 0.04 [a]
FCO	64.0 ± 0.1 [b]	16.0 ± 0.4 [b]	13.0 ± 0.1 [a]	3.03 ± 0.01 [b]
FCE	63.3 ± 0.1 [a]	16.2 ± 0.1 [b]	13.2 ± 0.1 [a]	3.21 ± 0.11 [c]
FCEG	63.4 ± 0.4 [a]	16.4 ± 0.1 [b]	12.5 ± 0.2 [a]	3.62 ± 0.06 [d]

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

Table 3. Half-bandwidth values of the $\nu_{as}CH_2$ and ν_sCH_2 bands of frankfurters.

Samples *	**Half-bandwidth**	
	$\nu_{as}CH_2$	**ν_sCH_2**
FP	26.5 ± 0.4^a	15.2 ± 0.1^a
FCO	29.6 ± 0.5^b	15.6 ± 0.2^b
FCE	31.7 ± 0.2^c	16.7 ± 0.4^c
FCEG	31.3 ± 0.2^c	16.9 ± 0.1^c

*For sample denominations see Table 1. Means ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

Highlights

Chia and olive oil were used, in different ways, to formulate reduced fat frankfurter

Lipid structure in frankfurters was affected by strategies to replace animal fat

Lipid structural features were correlated to processing loss and textural behaviour