**Oil bulking agents based on polysaccharide gels in meat batters: a Raman
spectroscopic study**

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Abstract

A Raman spectroscopic study was performed to determine protein and lipid structural properties in meat batter containing oil bulking agents as pork backfat replacers. Meat batters were prepared with pork backfat (MB-PF) or with a combination of olive oil, sodium alginate, CaSO_4 , sodium pyrophosphate and dextrin (MB-A/D) or inulin (MB-A/I) as fat replacer. Proximate composition, pH, cooking loss (CL), colour and texture were evaluated. MB-A/D and MB-A/I both showed lower ($P<0.05$) CL and a^* values, higher ($P<0.05$) L^* and b^* and higher ($P<0.05$) hardness and chewiness. MB-A/I showed the highest hardness and chewiness. Enhancement of β -sheet structure was observed in MB-A/D and MB-A/I, more so in MB-A/I. There was increased disorder in the oil acyl chains, which involve lipid–protein interactions, in both MB-A/D and MB-A/I. Structural characteristics in proteins and lipids may be associated with specific water and fat binding properties and textural characteristics of meat batters.

Keywords: lipid, protein, polysaccharide gel, meat batter, technological properties, structural characteristics.

1. Introduction

Recent years have seen growing interest in the consumption of foods that are perceived as healthier (including meat-based foods), and the trend is expected to continue over the next ten years. Because of their health implications, lipids are among the components that have received most attention for purposes of developing healthier foods. Their importance lies in the connection between the amount and type of fat in the diet and the risk of major chronic disease in our society, such as cardiovascular diseases, cancer and obesity. Because of their composition and prevalence of consumption, meat and meat products are among the foods that contribute most to dietary lipid intake. If we consider that in many cases the characteristics of meat lipids are not exactly those considered most recommendable, it is understandable that special interest should attach to the development of strategies designed to achieve quantitative and qualitative modification of their lipid profiles so as to bring them more into line with health recommendations (Jiménez-Colmenero, 2007). One of the strategies that has received most attention in this connection entails reformulation processes designed to replace animal fat with other fats of plant and/or marine origin which comply better with health recommendations. These fats have been incorporated in meat products in liquid and solid forms, encapsulated, or as oil-in-water emulsions (Jiménez-Colmenero, 2007). Strategies for incorporation of healthy oils in a gel-like matrix to form oil bulking agent (in which this new ingredient acts as an animal fat replacer) could offer new possibilities for improving the fat content of meat products. In this regard, healthy oils in a konjac matrix have been used to improve fat content in meat products (Ruiz-Capillas, Triki, Herrero, Rodríguez-Salas, & Jiménez-Colmenero, 2012; Salcedo-Sandoval, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, 2013). With similar purposes, our group recently developed two different polysaccharide gel matrices

containing olive oil, prepared using mixed biopolymer systems of alginate with inulin or dextrin **with optimal characteristics for use as animal fat replacers** (Herrero, Carmona, Jiménez-Colmenero, & Ruíz-Capillas, 2013). The technological and structural properties of these new oil bulking agents highlight the possibilities offered by the use of such matrixes as potential fat replacers in the development of meat products with improved fat content.

To properly develop healthier meat products of this kind, it is essential to examine aspects of reformulation processes that affect technological properties (water and fat binding properties, colour, texture, etc.) and to gain an understanding of the complex relationship between these properties and the different structural characteristics of the components of the meat matrix. Further investigation of this structure/function relationship would help to elucidate the mechanisms that act on them and to establish criteria for adjusting them, and criteria for assessing aspects relating to factors of processing and composition and their impact on various physicochemical properties of the complex meat system. In this context, Raman spectroscopy offers a powerful tool with which to address such questions. As a technique it has numerous advantages, among them the fact that it is a direct (non-invasive) method capable of simultaneously furnishing structural and analytical information on the different components (proteins, lipids, etc.) of meat and their derivate, and moreover it requires only small sample amounts (Pedersen, Morel, Andersen, & Engelsen, 2003; Böcker, et al., 2007; Damez, & Clerjon, 2008; Herrero 2008 a, b; Herrero, Carmona, Jiménez-Colmenero, & Ruiz-Capillas, 2010; Yang, & Ying, 2011). In the literature there are several references about the use of Raman spectroscopy coupled to chemometrics (multivariate analysis) to **determine predict** different physico-chemical properties (protein solubility, apparent viscosity, water holding capacity, fatty acid composition, instrumental textural methods,

etc.) in meat systems ~~treated under different conditions of handling, processing and~~
~~storage through the changes of proteins, water and lipids~~ (Pedersen et al., 2003; Damez,
& Clerjon, 2008; Herrero 2008 a, b; Herrero, Carmona, Jiménez-Colmenero, & Ruiz-
Capillas, 2010). ~~On the other hand, Raman spectroscopy has been also used specifically~~
to study protein structural changes in meat batters induced by thermal treatment or by
salt, soy protein or cold-set binding agents addition (Herrero, Carmona, López-López,
& Jiménez-Colmenero, 2008c; Herrero, Carmona, Cofrades, & Jiménez-Colmenero
2008d; Herrero, Cambero, Ordóñez, de la Hoz, & Carmona, 2009). Some authors have
also demonstrated that Raman spectroscopy is a useful tool for obtaining direct
information on the secondary and tertiary structural changes occurring in proteins of
meat batters as a result of addition of different lipids (pork fat, soybean oil and dairy
butter) and thermal treatment (Shao, Zou, Xu, Wu, & Zhou, 2011). These studies
revealed some connections between the protein structure and the technological
properties of the product such as texture and water binding (Herrero et. al., 2008 c, d;
Herrero et al., 2009; Shao et al., 2011).

A better understanding of the structural changes induced in meat batters by
incorporation of polysaccharide gels as oil bulking agents to replace animal fat, and of
their relationship with some of the physico-chemical characteristics of the batters, could
be helpful in optimizing the development of healthier meat products. The primary aim
of this work was therefore to examine changes in physico-chemical properties (colour,
water and fat binding properties and textural characteristics) produced in meat batters by
utilization of oil bulking agents based on polysaccharide gels as animal fat replacers,
and changes in protein and lipid structures of these meat systems, using Raman
spectroscopy. The secondary aim was to elucidate the relationship between the physico-
chemical properties and the protein and lipid structures of these meat batters.

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 polysaccharide gels as oils bulking agents included: olive oil (13% SFA, 79% MUFA and 8% PUFA) (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain); sodium alginate (90% carbohydrates) (Tradissimo, TRADES S.A., Barcelona Spain); calcium sulphate (Panreac Química, S.A. Madrid, Spain), tetra-sodium pyrophosphate anhydrous (STP) (Manuel Riesgo, S.A. Madrid, Spain), inulin consisting mainly of chicory inulin (>90% inulin) with a molecular weight of 1650 g/mol (TRADES S.A., Barcelona Spain) and white maize dextrin (molecular weight average approximate between 10 and 20 glucose molecule per polymer) (CARGILL S.L.U- CTS Rubi, Barcelona, Spain).

Sodium chloride (Panreac Química, S.A. Barcelona, Spain) and sodium tripolyphosphate (ST) (Manuel Riesgo, S.A. Madrid, Spain) were also used as ingredients.

2.2. Preparation of olive oil bulking matrices based on polysaccharide gels.

Two different types of polysaccharide matrices with olive oil were considered (Herrero et al., 2013): a combination of olive oil with sodium alginate, CaSO₄, STP and dextrin (A/D) or inulin (A/I). These matrices were prepared by mixing with water (40 %) in a homogenizer (Thermomix TM 31, Vorwerk España M.S.L., S.C, Madrid) SA

(1%), CaSO₄ (1%), STP (1%) and dextrin (2.25%) or inulin (2.25%) to prepare A/D or A/I respectively. The mixtures were prepared at 1500 rpm for 20 seconds. Olive oil (55%) was gradually added to this mixture with the homogenizer in running (1500 rpm). Matrices of each type were stuffed into metal moulds under pressure to compact them and avoid air bubbles, and stored in a chilling room at 2 °C for 24 h until analysis.

The preparation of each type of oil bulking agent was performed by duplicate using two

metal moulds for each type of sample. ~~Each type of sample was prepared in duplicate.~~

These polysaccharide matrices with olive oil were used as pork backfat replacer in the formulation of the meat batters.

2.3. Preparation of meat batters.

Three different meat batters were prepared as reported in **Table 1**: a control meat batter formulated with meat and pork backfat (MB-PF) and two reformulated samples in which the pork backfat was replaced by an equal amount of oil bulking agents, A/D or A/I to formulate MB-A/D and MB-A/I, respectively. 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 polysaccharide gel-based olive oil bulking matrices (MB-A/I or MB-A/D, depending on the formulation), NaCl and sodium tripolyphosphate (previously dissolved in the added water) were added to the ground meat and the whole mixed again for 1 min. The rest of the additives, the pork backfat or the polysaccharide matrices with olive oil 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 4 min. The final batter temperature was below 12 °C in all cases. Portions of each meat batter (approximately 70 g) were placed in plastic containers

(diameter 3.5 cm, height 7 cm), which were hermetically sealed. They were then heated in a water bath at 70 °C for 30 min. Thermocouples connected to a temperature recorder were used to establish the thermal conditions required to reach an internal temperature of 70 °C (Yokogawa Hokushin Electric YEW, Mod. 3087, Tokyo, Japan). Samples were stored in a chilling room at 2 ± 2 °C until analyses. Each formulation was prepared in duplicate.

2.4. Proximate analysis and pH.

Moisture and ash contents of the cooked meat batters were determined (AOAC, 2000) in triplicate. Protein content was measured in triplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). Fat content was evaluated in triplicate (Bligh & Dyer, 1959).

The pH was determined on a Radiometer model PHM 93 pH-meter (Meterlab, Copenhagen, Denmark) at room temperature on homogenates in water in a ratio 1:10 (w/v) of meat batters, pork backfat and oil bulking agents. Six determinations were performed for each formulation.

2.5. Cooking loss

Immediately after the heating process (70 °C/30 min) reported above (section 2.3) the containers were opened and left to stand upside down for 50 min to release the exudate onto a plate that had been previously weighed. Water and fat binding properties were estimated as cooking loss (CL) and expressed as % of initial sample weight. Determinations were carried out four times.

2.6. Colour measurement.

Colour, CIE-LAB tristimulus values, lightness, L*; redness, a* and yellowness, b* of the cross-section of cooked samples were evaluated on a Chroma Meter CR-400 (Konica Minolta Business Technologies, Inc., Tokyo, Japan). Eight determinations were performed from each formulation.

2.7. Texture Profile Analysis.

Texture Profile Analysis (TPA) was performed in a TA.XTplus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Six cylindrical cores (diam = 20 mm, height = 20 mm) from each cooked meat batter formulation were axially compressed to 30 % of their original height (Bourne, 1978). Force-time deformation curves were obtained with a 5 kg load cell, applied at a crosshead speed of 1 mm/sec. Attributes were calculated 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; Chewiness (Cw): Hd x Ch x Sp (N x mm). Measurement of samples was carried out at room temperature.

2.8. FT-Raman spectroscopic analysis.

Various Raman spectra were measured and used for reference: spectra of heated homogenized raw meat, heated pork backfat, and heated polysaccharide matrices with olive oil (A/I and A/D). Heating was performed in a water bath at 70 °C for 30 min, as in the preparation of meat batters. Spectra of meat batters formulated according to **Table 1** (MB-PF, MB-A/I and MB-A/D) were also measured.

Portions (approximately 5 g) of the different samples were transferred to quartz cuvettes (ST-1/Q/10) (Teknokroma, Barcelona, Spain), which were filled to reach 1 cm

sample length. For each sample 1500 scans were recorded. This procedure was carried out in triplicate, giving a total of 4500 scans per sample. Measurements were performed on three samples from each meat batter formulation. Spectra were excited with the 1064 nm Nd: YAG laser line and recorded on a Bruker RFS 100/S FT-spectrometer. The scattered radiation was collected at 180° to the source, and frequency-dependent scattering of the Raman spectra was corrected by multiplying point by point with $(\nu_{\text{laser}}/\nu)^4$. The influence of the optics on the spectrometer was eliminated by means of Opus 2.2 software Raman correction command (Bruker, Karlsruhe, Germany). Reported frequencies are accurate to $\pm 0.5 \text{ cm}^{-1}$, as deduced from frequency standards measured in the spectrometer. Raman spectra were resolved at 4 cm^{-1} resolution with a liquid nitrogen-cooled Ge detector. The samples were illuminated by laser power at 300 mW.

Raman spectra were processed using Opus 2.2 (Bruker, Karlsruhe, Germany) and Grams/AI version 9.1 (Thermo Electron Corporation, USA) software. For intensity measurements, baselines were considered as straight lines drawn tangentially between the intensity minima occurring on either side of each band

2.9. Statistical analysis.

Analysis of variance (ANOVA one way) and Tukey's multiple range test were carried out to evaluate the statistical significance ($P < 0.05$) of the effect of meat batter 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. Pearson's product moment correlation (R) was performed to determine the relationships between technological properties (cooking loss, colour and texture parameters) and Raman spectroscopy data. Statistical analysis was performed using Statgraphics Plus version 5.0.

3. Results and Discussion

3.1. Proximate analysis and pH.

Proximate analysis of cooked meat batters (**Table 2**) revealed some differences according to the type of formulation (**Table 1**). Protein concentration in MB-PF was similar ($P>0.05$) to MB-A/D but higher ($P<0.05$) than MB-A/I. Both the reformulated meat batters (MB-A/I and MB-A/D) contained similar ($P>0.05$) protein levels. These differences were attributed partly to the fact that the protein in the MB-PF was from meat raw materials (meat and pork backfat), while the other two samples (MB-A/I and MB-A/D) did not contain small amount of protein from pork backfat, and oil bulking agents did not contribute to the protein level. Moisture and ash contents varied between 59.2-62.2% and 3.07-3.59%, respectively, with the lowest proportion in the all animal fat sample (MB-PF) in both cases. Fat content ranged between 16.95% and 20.06% (**Table 2**), with the highest ($P<0.05$) value in the MB-PF sample (all pork fat). These results were consistent with the fact that the ingredients added to replace pork backfat (A/D and A/I) contained a smaller proportion of fat (and more moisture) than the pork backfat. Meat batters with polysaccharide matrices (containing 55 % olive oil) replacing pork backfat contained around 13.5 g of olive oil per 100 g of product. This suggests that depending on the nature and composition of the lipidic components included in the polysaccharide matrix (in this case olive oil), this strategy could be used to improve the fatty acid profiles of meat products and promote intake of significant amounts of such bioactive compounds.

The pH values (**Table 2**) in the MB-PF samples were lower ($P<0.05$) than in any of the reformulated meat batters (MB-A/D and MB-A/I). This is possibly because the pork backfat used in the MB-PF formulation had a lower ($P<0.05$) pH (5.96 ± 0.10) than the polysaccharide matrices with olive oil (A/I: 7.6 ± 0.03 ; A/D: 7.7 ± 0.04).

3.2. Cooking loss

The ability of the protein matrix to bind water and fat upon heating is crucial in the manufacture of processed meat products. These properties determine the final cooking yield, but also the texture and other attributes defining final product quality. The effect of heating on water and fat binding was influenced by the formulation. Results showed that cooking loss was **lowest** ($P < 0.05$) in meat batters formulated with polysaccharide matrices containing olive oil as compared with all-animal-fat sample (MB-PF) (**Table 2**). The type of oil bulking agent did not affect ($P > 0.05$) cooking loss of meat batters (**Table 2**). These differences in cooking losses could account for the lower moisture content and higher protein and fat proportions of MB-PF sample (**Table 2**). In the reformulated meat batters with added olive in polysaccharide matrices (MB-A/D and MB-A/I), part of the water and fat present in the product was embedded and retained in these matrices, so that less was released during cooking (**Table 2**). Similar behaviour has been reported in low-fat pork batters using polysaccharides such as konjac glucomannan in the form of a gel as fat replacer (Fernández-Martín, López-López, Cofrades, & Jiménez Colmenero, 2009). However, it should be noted that there are conflicting results about the effect in cooking loss of meat batters in which animal fat was replaced by vegetable oils. Some authors indicated that cooking loss is greater in frankfurters prepared with vegetable oils than with animal fat (Paneras & Bloukas, 1994; Ambrosiadis, Vareltzis, & Georgakis, 1996; Lopez-Lopez, Cofrades, Ruiz-Capillas, & Jimenez-Colmenero, 2009). On the contrary, other authors reported that cooking loss in meat batters formulated with oils as fat replacer decreased or it was not affected (Shao et al., 2011; Delgado-Pando, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2010; Álvarez, Delles, Xiong, Castillo, Payne, & Laencina, 2011; Salcedo-Sandoval, et al., 2013). In this way, it has been commented that the effect in cooking loss was affected by type of oil, the

characteristics of the meat systems (in terms of relation protein/moisture) and the technological strategy used to incorporate the oil combination.

3.3. Colour measurement.

Meat batters colour was affected by formulation (**Table 3**). Values of L^* were lowest ($P<0.05$) in MB-PF sample (all pork fat). Replacement of pork backfat with oil bulking agents increased ($P<0.05$) b^* values and reduced ($P<0.05$) redness (a^*) (**Table 3**). This behaviour may be related to the difference in colour between the pork backfat and the polysaccharide matrices with oil, whose colour was more yellowish-green due to the olive oil (Jiménez-Colmenero, Herrero, Pintado, Solas, & Ruiz-Capillas, 2010). These results are consistent with reports in which the replacement of pork backfat by vegetable oils increased lightness and reduced redness of cooked sausages (Bishop, Olson, & Knipe, 1993; López-López et al., 2009; Jiménez-Colmenero et al., 2010).

3.4. Texture Profile Analysis (TPA).

Texture profile analysis parameters are shown in **Table 3**. Textural behaviour was affected by formulation. Reformulated meat batters made with any of the different polysaccharide matrices with olive oil presented higher ($P<0.05$) hardness and chewiness than those made with all pork backfat (**Table 3**). Springiness and cohesiveness were similar ($P>0.05$) in all the meat batters studied. This behaviour may be due to the effect of either two factors: differences in products composition and the presence of oil bulking agents. Given that the protein/moisture ratio was higher in MB-PF than in MB-A/D and MB-A/I samples, a more "effective" protein concentration should help to form the gel/emulsion matrix in that sample, and so the structures should be harder/firmer. The fact that the behaviour was different would appear to be due to the

310 presence of the oil bulking agent. Our results suggest that by reformulating frankfurters
311 with polysaccharide gels based on konjac glucomannan and containing a combination of
312 vegetable and marine oils as animal fat replacers, a product can be achieved that is
313 harder and chewier than a control frankfurter formulated with pork backfat (Salcedo-
314 Sandoval et al., 2013). Similarly, reduced-fat frankfurters in which pork backfat is
315 replaced by an equal amount of a mixed konjac/gellan gum gel are generally harder and
316 chewier than regular high-fat frankfurters (Lin & Huang, 2003). It has been reported
317 that meat batters prepared with soybean oil (added directly to meat) showed greater
318 hardness, springiness, cohesiveness, chewiness and resilience than those made with
319 pork fat (Shao et al., 2011). Some authors have also observed increased firmness in
320 cooked meat products prepared with pre-emulsified vegetable or fish oil as animal fat
321 replacers (Delgado-Pando et al., 2010; Jiménez-Colmenero et al., 2010; Youssef &
322 Barbut, 2011).

323 The difference in textural behaviour observed between meat batters formulated
324 with pork backfat and batters containing these polysaccharide matrices with added olive
325 oil shows the extent to which the composition of lipid fraction can determine the
326 textural characteristics of final cooked meat products.

328 *3.5. FT-Raman spectroscopic analysis*

329 *Protein structure:* Spectral region 800-1800 cm^{-1} was analysed to determine how
330 meat protein structure was affected by changes resulting from reformulation. To that
331 end the corresponding spectrum of the heated polysaccharide matrix/oil (A/D or A/I)
332 was subtracted from the reformulated meat batters by zeroing the bending $=\text{CH}$ band
333 located close to 1267 cm^{-1} , which is attributed to lipid molecules (Zou et al., 2009).
334 Similarly, heated pork backfat spectrum was subtracted from the control meat batters

following the same criteria in order to eliminate any spectral influence of lipids in this spectral region (800-1800 cm^{-1}). The Phe ν -ring band located near 1003 cm^{-1} was used as an internal standard to normalize the spectra as it has been reported to be insensitive to the micro-environment (Herrero, 2008a, b; Herrero et al., 2010). The visible bands were assigned to vibrational modes of peptide backbone or amino acid side chains by comparing Raman spectra of model polypeptides or monographs of Raman spectra of proteins (Herrero, 2008a, b; Herrero et al., 2010). The intensity values of Raman bands from various atomic groups were determined after spectral normalization. Protein secondary structures were determined as percentages of α , β , turns and unordered conformations (Alix, Pedanou, & Berjot, 1988).). For this purpose, the water spectrum was first subtracted from the spectra following the same criteria as described in other literature references (Alix et al., 1988).

The amide I band (1620 -1720 cm^{-1}) was used to study the structural changes occurring in the meat protein when pork backfat was replaced with olive oil polysaccharide matrices in the formulation (**Fig. 1**). This band includes mainly C=O stretching and, to a lesser extent, N-H in-plane bending of peptide groups (Herrero, 2008a, b; Herrero et al., 2010). The frequency and intensity changes in this Raman band were mainly indicative of changes in the secondary structure and variations in local environments of meat proteins. A comparison of amide I band of control meat batter (MB-PF) and reformulated meat batters revealed a shift of the absorption maximum from 1666 to 1668 cm^{-1} in MB-A/D and from 1666 to 1669 cm^{-1} in MB-A/I. This spectral change is indicative of changes in meat protein structure consisting of increased β -sheet structure content resulting from the use of these olive oil bulking agents as animal fat replacers.

Analysis of amide I spectra profiles provided a quantitative estimation of the percentages of secondary structure for the different meat batters studied (**Table 4**). There was a significant decrease ($P<0.05$) in α -helix accompanied by a significant increase ($P<0.05$) in β -sheet and turn structure after animal fat was replaced by polysaccharide matrices with added oil (**Table 4**). These protein structural changes were more pronounced ($P<0.05$) in the matrices containing inulin (MB-A/I). This is consistent with the amide I spectra changes described above (**Fig. 1**) and with literature references reporting that replacement of animal fat with olive oil-in-water emulsion in the preparation of frankfurter is accompanied by an increase in β -sheet structure (Carmona, Ruiz-Capillas, Jiménez-Colmenero, Pintado, & Herrero, 2011). Enrichment of aggregated intermolecular β -sheets, as observed in the present work, has been associated with the formation of a denser network providing more firmness. Previous morphological studies have indicated that frankfurters formulated with protein-stabilized oil-in-water emulsion presented more cavity formation or narrow particle size distribution, generally due to smaller fat globules (Jiménez-Colmenero et al., 2010; Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, Triki, & Jimenez-Colmenero, 2011). This suggests that there is a larger surface area covered by a denser protein matrix structure; which in turn could be attributable to greater aggregation of intermolecular β -sheets, thus resulting in firmer products.

The observed changes in meat protein secondary structure due to reformulation with oil bulking agents as animal fat replacers were accompanied by changes in the cooking loss and texture of meat batters. For instance, there was a positive correlation ($R= 0.89$, $P<0.005$) between α -helix content and cooking loss and a negative correlation ($R= -0.90$, $P<0.005$) between β -sheet structure and cooking loss. These results suggest that the observed protein secondary structural changes resulting from reformulation

could be decisive for the water and fat binding properties of the protein network (Tables 2 and 4). In addition, there was a significant positive correlation between β -sheet structure and hardness ($R = 0.92$, $P < 0.05$). Similarly, other authors have reported a positive 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).

The Raman bands at 1450 cm^{-1} were assigned to the CH_2 and CH_3 bending vibrations (Table 4). A reduction in the intensity of this band may result from hydrophobic interactions involving aliphatic residues (Herrero, 2008a, b; Herrero et al., 2010). Our results showed a significant ($P < 0.05$) decrease of the 1450 cm^{-1} band from the control (MB-PF) to the reformulated meat batters (MB-A/D and MB-A/I) (Table 4), suggesting that there may have been an increase in hydrophobic interactions in the samples reformulated with polysaccharides matrices with oil as a fat replacer. This decrease was more pronounced in MB-A/I (Fig. 2). These results suggest that the protein cross-linking could be due largely to this type of hydrophobic contact between aliphatic protein side chains, which tends to be greater when inulin is present in the oil polysaccharide matrix.

Lipid structure. Raman spectroscopy also provides information for structural characterization of lipid molecules. Spectral region $2800\text{-}3000\text{ cm}^{-1}$ was analysed to examine the lipid structure. In order to eliminate any spectral influence of water in the samples in this spectral region ($2800\text{-}3000\text{ cm}^{-1}$), the spectral contribution of water was subtracted from the sample spectra (Alix et al., 1988). The water-free spectra of the

samples were then appropriately subtracted using the heated raw meat spectrum, based on elimination of the Phe v-ring band by means of a subtraction factor so that the intensity of this Raman band near 1003 cm^{-1} is not visible. In this way, spectral influence of protein was eliminated.

The lipid bending =CH band located near 1267 cm^{-1} was used as an internal standard to normalize the resulting spectra (Zou et al. 2009).

Typical normalized FT-Raman spectra in the $2800\text{-}3050\text{ cm}^{-1}$ region of the different meat batter are shown in **Fig. 2**. The spectra showed bands that are characteristic of symmetric and asymmetric C-H stretching vibrations of methyl and methylene groups in aliphatic molecules (Muik, Lendl, Molina-Díaz, & Ayora-Cañada, 2005; Zou et al. 2009; Herrero et al., 2010). Thus, the CH_2 asymmetric stretching band appears at 2898 cm^{-1} , the CH_3 symmetric stretching band at 2932 cm^{-1} and the CH_2 symmetric stretching motion close to 2853 cm^{-1} . Another Raman band at 3007 cm^{-1} attributable to *cis*-olefinic group =C-H stretching vibration is clearly visible. It has been reported that the peak height intensity ratios $\text{Iv}_s\text{CH}_2/\text{Iv}_{as}\text{CH}_2$ ($\text{I}_{2850}/\text{I}_{2880}$) and $\text{Iv}_s\text{CH}_3/\text{Iv}_{as}\text{CH}_3$ ($\text{I}_{2935}/\text{I}_{2880}$) provide useful indices for gauging lipid packing effects and determining relative order/disorder of the intermolecular lipid chain (Levin, & Lewis, 1990). In particular, the $\text{Iv}_s\text{CH}_2/\text{Iv}_{as}\text{CH}_2$ index reflects primarily interchain interactions whereas the $\text{Iv}_{as}\text{CH}_2/\text{Iv}_s\text{CH}_3$ intensity ratio measures effects originating from changes in intrachain *trans*/*gauche* isomerization superimposed on the chain-chain interactions. Non-significant changes in intensity ratio $\text{Iv}_s\text{CH}_3/\text{Iv}_{as}\text{CH}_3$ were observed (**Table 4**), while $\text{Iv}_s\text{CH}_2/\text{Iv}_{as}\text{CH}_2$ was lower ($P<0.05$) in the meat batter made with polysaccharide matrices containing olive oil as animal fat replacer (**Table 4**). These significant differences suggest that there was more lipid acyl chain disorder in the reformulated meat batter (MB-A/D and MB-A/I) than in the control made with pork backfat (MB-

PF). This disorder may be ascribed to the fact that protein chains are inserted to some extent between the acyl chains of the oil, involving lipid–protein interactions, these being more pronounced ($P < 0.05$) in meat batter formulated with polysaccharide matrices containing olive oil as an animal fat replacer. The results showed a negative correlation ($R = -0.89$, $P < 0.05$) between $I_{\nu_s \text{CH}_2}/I_{\nu_{\text{as}} \text{CH}_2}$ and β -sheet content. More acyl chains disorder or lipid-protein interaction involves more β -sheet content in reformulated samples with polysaccharide matrices with oil as fat replacer. Also, a positive correlation ($R = 0.96$, $P < 0.005$) was found between $I_{\nu_s \text{CH}_2}/I_{\nu_{\text{as}} \text{CH}_2}$ and cooking loss. This correlation suggests that disordering in oil acyl chains or lipid-protein interaction, resulting from reformulation, could be decisive for the water and fat binding properties of the final meat product

Conclusions

This article shows that Raman spectroscopy is a powerful technique for obtaining direct information on the changes taking place in the protein and lipid structure of meat systems, such as meat batters, as a result of a reformulation process based on used of oil bulking agents based on polysaccharide matrices as animal fat replacers. For instance, increases of β -sheet structure content and hydrophobic interactions were observed in response to reformulation. These protein structural changes may be a result of protein cross-linking, which could be due to hydrophobic contact between aliphatic protein side chains. This is more pronounced when inulin is present in the oil bulking agent that is used as an animal fat replacer.

The protein structural characteristics seem to play an essential role in some technological properties of meat batters. Replacement of pork backfat with polysaccharide matrices containing olive oil produces an increase of β -sheet structure accompanied by

enhanced water and fat binding properties and stronger textural characteristics, specifically hardness and chewiness.

Also, spectral results revealed changes in lipid structure resulting from increased lipid acyl chain disorder in samples containing polysaccharide matrices with olive oil as a fat replacer. This suggests that there were more lipid–protein interactions in reformulated meat batters, which could be related to enhanced water and fat binding properties in reformulated samples.

Information regarding how these reformulated processes affects structures of the main components (protein and lipids) in these meat derivatives and how this correlates with the technological properties of the finished product, could be helpful in optimizing development and formulation conditions of healthier meat products with optimal lipid content.

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Table 1. Formulation (g) of meat batters.

Samples*	Meat	Pork backfat	polysaccharides matrices with olive oil		Water
			A/D	A/I	
MB-PF	700	245			32
MB-A/D	700	-	245		32
MB-A/I	700	-		245	32

Additives added to all samples: 2.0 g/100 g NaCl; 0.30 g/100 g sodium tripolyphosphate.
*Meat batter formulated with pork backfat (MB-PF) or a combination of olive oil, sodium alginate, CaSO₄, sodium pyrophosphate and dextrin (MB-A/D) or inulin (MB-A/I) as fat replacer.

Table 2. Proximate analysis, pH and cooking loss (CL, %) of meat batters.

Samples*	Moisture	Fat	Protein	Ash	pH	CL (%)
MB-PF	59.20 ± 0.24 ^b	20.06± 0.25 ^a	18.05 ± 0.84 ^a	3.07 ± 0.01 ^b	5.99 ± 0.09 ^b	9.83±0.48 ^a
MB-A/D	62.23 ± 0.18 ^a	17.39± 0.22 ^b	16.65 ± 0.46 ^{ab}	3.43 ± 0.04 ^a	6.39 ± 0.03 ^a	2.49±0.45 ^b
MB-A/I	61.95 ± 0.19 ^a	16.97± 0.29 ^b	16.15 ± 0.44 ^b	3.59 ± 0.06 ^a	6.37 ± 0.01 ^a	2.70±0.59 ^b

*Meat batter formulated with pork backfat (MB-PF) or a combination of olive oil, sodium alginate, CaSO₄, sodium pyrophosphate and dextrin (MB-A/D) or inulin (MB-A/I) as fat replacer.
Means ± standard deviation. Different letters in the same column indicate significant differences (P<0.05).

Table 3. Colour parameters (L* lightness, a* redness and b* yellowness) and textural profile analysis (TPA) parameters (hardness, springiness, cohesiveness, chewiness) of meat batters.

Samples*	L	a*	b*	Hardness (N)	Springiness (mm)	Cohesiveness (dimensionless)	Chewiness (N×mm)
MB-PF	57.70 ± 0.78 ^b	8.39 ± 0.71 ^a	6.22 ± 0.34 ^b	16.12 ± 0.22 ^c	4.86 ± 0.31 ^a	0.49 ± 0.04 ^a	39.43 ± 3.06 ^c
MB-A/D	60.52 ± 0.55 ^a	4.96 ± 0.83 ^b	10.06 ± 0.93 ^a	17.93 ± 0.48 ^b	5.04 ± 0.42 ^a	0.55 ± 0.03 ^a	48.56 ± 2.34 ^b
MB-A/I	59.87 ± 0.33 ^a	4.73 ± 0.79 ^b	10.81 ± 0.39 ^a	19.71 ± 0.33 ^a	4.92 ± 0.23 ^a	0.53 ± 0.09 ^a	53.40 ± 2.15 ^a

*Meat batter formulated with pork backfat (MB-PF) or a combination of olive oil, sodium alginate, CaSO₄, sodium pyrophosphate and dextrin (MB-A/D) or inulin (MB-A/I) as fat replacer.

Means ± standard deviation. Different letters in the same column indicate significant differences (P<0.05).

Table 4. Percentages of α -helix, β -sheet, turns and unordered protein structures, relative intensity of δ CH ($I_{\delta\text{CH}}/I_{\text{Phe}}$), $I_{\text{vsCH}_2}/I_{\text{vasCH}_2}$) and $I_{\text{vsCH}_3}/I_{\text{vasCH}_2}$ of the meat batters.

Samples [*]	α -helix	β -sheet	turns	unordered	$I_{\delta\text{CH}}/I_{\text{Phe}}$	$I_{\text{vsCH}_2}/I_{\text{vasCH}_2}$	$I_{\text{vsCH}_3}/I_{\text{vasCH}_2}$
MB-PF	27.0 $\pm$ 0.6 ^a	41.1 $\pm$ 0.9 ^c	19.5 $\pm$ 0.8 ^c	12.4 $\pm$ 0.7 ^a	3.54 $\pm$ 0.3 ^a	1.07 $\pm$ 0.02 ^a	0.95 $\pm$ 0.03 ^a
MB-A/D	20.5 $\pm$ 0.8 ^b	46.6 $\pm$ 0.7 ^b	20.4 $\pm$ 0.6 ^b	12.6 $\pm$ 0.9 ^a	2.98 $\pm$ 0.2 ^b	0.92 $\pm$ 0.03 ^b	0.94 $\pm$ 0.01 ^a
MB-A/I	14.1 $\pm$ 0.7 ^c	50.0 $\pm$ 0.6 ^a	22.1 $\pm$ 0.4 ^a	13.7 $\pm$ 1.1 ^a	1.95 $\pm$ 0.6 ^c	0.91 $\pm$ 0.02 ^b	0.95 $\pm$ 0.02 ^a

*Meat batter formulated with pork backfat (MB-PF) or a combination of olive oil, sodium alginate, CaSO₄, sodium pyrophosphate and dextrin (MB-A/D) or inulin (MB-A/I) as fat replacer.

Means $\pm$ standard deviation. Different letters in the same column indicate significant differences (P<0.05).

Figure(1)

Fig. 1.

Fig. 2.