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Raman Spectroscopic Evaluation of Meat Batter Structural Changes Induced by Thermal Treatment and Salt Addition

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Raman spectroscopy, texture, proximate composition, and water binding analysis were carried out to evaluate the effect of thermal treatment and/or salt addition to meat batter. For this purpose, different meat batters were elaborated: control meat batter (no salt) and meat batters with low (1.0%) and high (2.5%) NaCl content with and without thermal treatment (70 °C/30 min). Increase ($P < 0.05$) in penetration force and hardness upon heating was observed. Results also showed hardness increasing ($P < 0.05$) as a function of salt addition in heated meat batter. Raman spectroscopy analysis revealed a significant ($P < 0.05$) decrease in α-helix content accompanied by an increase ($P < 0.05$) in β-sheets resulting from heating. Significant ($P < 0.05$) correlations were found between these secondary structural changes in meat proteins and water binding and textural properties of meat batter. In this way, a significant correlation was found between β-sheets, salt content, hardness, and chewiness in heated samples.

KEYWORDS: Meat batter; salt content; thermal treatment; Raman spectroscopy; texture

INTRODUCTION

Sodium intakes in most developed countries greatly exceed physiological requirements. High salt intake has been related to high blood pressure, one of the three major risk factors of cardiovascular diseases (*1, 2*). Since over 20−30% of dietary salt comes from meat products (*2, 3*), there is a growing interest among consumers and processors in reducing the use of salt (minimizing sodium) in meat processing.

Sodium chloride affects the flavor, texture, and shelf life of meat products. When finely chopped, the meat is reduced to a fine particulate state in the presence of salt, which induce swelling, water binding, and partial extraction of salt-soluble protein components. Subsequently, the thermal treatment induces the establishment of a stable gel network protein matrix (*4*). The properties of these meat gels in comminuted systems depend on several factors, including salt levels used in the formulation. Product development and studies concerning the reduction of salt should involve more basic knowledge that it may have on technological functions and their relationship with muscle protein structural properties (*2*).

The compositional change (salt reduction) effect on meat systems and its consequences on thermal gelation processes have been evaluated on the basis of properties such as water and fat binding, texture, microstructure, and so forth (*1, 2, 5*). The changes in meat protein structures upon thermal processing can be analyzed by direct and indirect structural measurements (*6*). There are several indirect studies in the literature references that undertake these changes through the modifications in salt-soluble protein interactions produced by heating (*4*). The meat gelation process had been studied by electrophoretic analysis of protein extracts in order to evaluate the types and proportion of proteins involved in gelation and their relationship to the rheological properties of the resulting gels (*7–9*). Differential scanning calorimetry has been used to measure salt effect on conformational protein changes in heated pork meat batters (*10*). In addition, Fourier transform infrared (FT-IR) microspectroscopy has been used to determine salt- and heat-induced structural changes in beef and pork muscle (*11–15*). However, to better control the protein structural changes in meat products caused by thermal treatment and/or salt addition, the process should be monitored by direct structural measurement, without pretreating the sample. In this way, low-field nuclear magnetic resonance (NMR) has been used in pork muscle to determine the structural changes that occur during heating and upon salt addition (*12, 14, 15*). However, low-field NMR mainly gives specific information about water mobility and water distribution in meat.

An alternative to this can be the use of Raman spectroscopy, which is a noninvasive spectroscopic technique overcoming

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most of the above drawbacks and providing in situ information about the structure of proteins (secondary and tertiary), water, and lipids (*16–22*), which are the main components of meat. This spectroscopic technique has been used to study the substructure of myosin isolated from rabbit (*23*) and the effect of inorganic salts ($CaCl_2$, $MgCl_2$ and LiBr) on myosin solutions (*24*). Conformational studies on thermal gelation of surimi have also been undertaken using Raman spectroscopy (*25, 26*). In addition, recently, some Raman spectroscopy studies have been carried out to examine the meat protein structural changes that occur upon the addition of cold-set binding agents to meat systems (*27, 28*). Moreover, Raman microspectroscopic studies on pork muscle fiber tissue subjected to different processing parameters have been performed (*29*). However, as far as we know, no Raman spectroscopic study has been performed to determine the protein structural changes that occur in meat products as effects of thermal treatment and/or salt addition, which are the more useful process conditions in the elaboration of meat products.

A clear understanding of the key of the structural properties that influence the quality characteristics of meat products will facilitate the development of high-quality low-salt products. Therefore, the aim of this work was to study protein structural changes by Raman spectroscopy that occur on meat batters as affected by thermal treatment and salt content. These protein structural changes have also been correlated with modifications in water binding and textural properties of the meat batter.

MATERIALS AND METHODS

Meat Preparation and Additives. Fresh pork meat (3 kg) was trimmed of fat and connective tissue and passed through a grinder (Mainca, Granollers, Spain) with a 0.6 cm plate. Sets of aproximately 800 g were vacuum packed and stored at 2 ± 2 °C until the next day to prepare the different meat batters.

Preparation of Meat Batter. Three different meat batters were formulated: control meat batter (C), prepared with 10% added water and without NaCl addition (Panreac Quimica, S.A. Barcelona, Spain); low salt meat batter (LS), manufactured containing 10% added water and 1% sodium chloride; and finally high salt meat batter (HS), with 10% added water and 2.5% NaCl. The preparation procedure was as follows: meat was homogenized for 1 min in a chilled cutter (Stephan Universal Machine UM5, Stephan u. Sóhne GmbH & Co., Hameln, Germany). Then, water and NaCl, dissolved in chilled water according to formulation, were added, and the whole was mixed again for 1 min. Finally, the whole meat batter was homogenized under vacuum conditions for 1 min. Mixing time was standardized to 3 min, and the final 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), hermetically sealed. Then, the plastic containers were centrifuged at 2500*g* and 3 °C for 15 min (Multifuge 3 L-R, Kendro Laboratory Products GmbH, Hanau, Germany) to homogenize them and eliminate any air bubbles. Two different treatments of each meat batter formulation placed in plastic containers were performed. Half of the containers from each meat batter formulation were chilled at 2 °C and analyzed as fresh (unheated) batters. The rest of the containers in each case were heated in a water bath at 70 °C for 30 min. The internal temperature was measured using thermocouples connected to a temperature recorder (Yokogawa Hokushin Electric YEW, Mod. 3087, Tokyo, Japan). Both unheated (CU, LSU, and HSU) and heated (CH, LSH, and HSH) samples were stored in a chilling room at 2 ± 2 °C until analyses.

Proximate Composition. Moisture and ash contents of the meat batters were determined (*30*) in triplicate. Protein content was measured in triplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI).

Fluid Released. Immediately after the heating process previously reported (70 °C/30 min), the containers were opened and left to stand upside down (for 50 min) to release the exudate onto a plate previously weighted. Water binding properties were estimated as fluid released (FR) and expressed as percent of initial sample weight. Determinations were carried out four times.

Penetration Test. Texture analysis based on a penetration test was performed on unheated and heated samples once the samples had attained room temperature. The test was performed using a 5 mm diameter cylindrical stainless steel plunger attached to a 50 N cell connected to the crosshead of TA-XT.plus texture analyzer (Texture Technologies Corp., Scarsdale, NY). Force-deformation curves were obtained at 0.2 mm/s crosshead speed. Penetration force (PF in N) was taken as the force required to penetrate 5 mm. Determinations were carried out eight times.

Texture Profile Analysis. For heated samples, texture profile analysis (TPA) was performed in a texture analyzer according to Bourne (*31*). Four sample cores (diameter 2.5 cm, height 2.0 cm) were axially compressed to 30% of their original height. Force−time deformation curves were derived with a 250 N load cell applied at a constant crosshead speed of 0.8 mm/s. 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) of sample recovery after the first compression; and chewiness (Cw), Hd × Ch × Sp (N·mm). Measurement of samples was carried out at room temperature.

Raman Spectroscopy Analysis. Portions of the different meat batter formulated were transferred to glass tubes (5 cm height and 5 mm i.d.; Wilmad Glass Co., Inc., Buena, NJ) to fill ∼1 cm length (*27*). For each sample, 1500 scans (about 45 min) were recorded. This procedure was carried out in triplicate, resulting in a total of 4500 scans per sample. Measurements were performed in three samples for each system. 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 that occurs with this spectrometer was corrected by multiplying point by point with $(\nu_{laser}/\nu)^4$. Reported frequencies are accurate to ± 0.5 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, thermostatted at 15−20 °C, were illuminated by laser power at 300 mW. Raman spectra were processed using Opus 2.2 (Bruker, Karlsruhe, Germany) and Grams/AI (Thermo Electron Corporation, Waltham, MA) software.

The Phe ν-ring band located near 1003 cm^{-1} was used as internal standard to normalize the spectra as it has been reported to be insensitive to the microenvironment (*16, 21, 22*). Assignment of the visible bands to vibrational modes of peptide backbone or amino acid side chains was carried out by comparing Raman spectra of model polypeptides or monographs of Raman spectra of proteins (*16, 27*). 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 (*32*). In this connection, the method used here is based on the frequency measurements at the intensity maximum of the amide I band and at the two ends in the half-bandwidth segment of this band. These frequency values are introduced into the parametric equation described by Alix et al. (*32*), which permits to obtain accurate quantitative estimation of secondary protein conformation. For this purpose, the water spectrum was previously subtracted from the spectra by following the same criteria as those described in other literature reference works (*32*).

Statistical Analysis. Analysis of variance (one-way ANOVA) was performed taking as covariate the thermal treatment for each salt concentration. Moreover, ANOVA was performed with salt content as the factor for each thermal treatment. To check the normal distribution of samples, the Shapiro−Wilks test was applied. Kruskal−Wallis test was used to test samples that did not fit the normal distribution. Duncan's test for multiple mean comparisons and Pearson product moment correlation (*R*) were performed to determine the relationships between data obtained by water binding, texture, and Raman spectroscopy data. Statistical analysis was carried out using a Statgraphics Plus version 5.0.

Table 1. Fluid Released (%) of Heated Meat Batters[a]

sample	fluid released (%)
CH	17.09 ± 0.72 a
LSH	6.93 ± 0.14 b
HSH	2.31 ± 0.39 c

[a] CH, heated control meat batter; LSH, heated low salt (1%) meat batter; HSH, heated high salt (2.5%) meat batter. Different letters (a, b, c) in the same column indicate significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Proximate Composition. Samples compositions are consistent with meat batter formulations. The composition of control meat batter was 18.6% protein, 77.2% moisture, and 0.9% ash. For low and high salt, the compositions were 19.1 and 18.5% protein, 75.1 and 76.2% moisture, and 1.9 and 3.3% ash, respectively.

Fluid Released. The ability of protein matrix to bind water upon heating is crucial in the manufacture of processed meat products. These properties determine the final cooking yield and also the texture and other features that constitute the final products' quality. The effect of heating process on water binding was affected by formulation. As expected, the higher the salt content the higher were the water binding properties (**Table 1**). Unlike the control sample (no salt) showing the highest FR values (17.1%), high salt meat batters exhibited the lowest ones (2.3%). The increasing of salt-soluble protein due to salt addition increased hydration and water binding properties in processed meats. Our results showed a significant positive correlation ($R = 0.944$, $P < 0.0005$) between water binding properties and salt content. According to the results obtained here, direct relationship between water binding properties and salt content has been reported (*2, 5, 33*).

Penetration Test. Penetration test was carried out in order to compare the rheological behavior of unheated and heated meat protein matrix as affected by salt content. Penetration force was affected by thermal treatment but not by formulation. All unheated samples showed similar PF values ($P > 0.05$) in the 0.195−0.221 N range. Heating process generated an increase in PF ($P < 0.05$), which varied from 3.3 to 3.6 N. The absence of differences in uncooked samples caused by salt can be due to the fact that gel formation does not occur in these conditions. In heated samples, the thermal gelation of meat protein explain the PF increasing. Heated samples do not reveal PF variations caused by salt content, as shown in unheated samples. It has been reported that, in muscle-based foods, the increasing of salt-soluble protein due to salt addition, followed by heating process, results in formation of a stable gel network (*4*). However, such phenomenon is not reflected in the results of PF, which can be attributable to the specific measurement conditions. Although in uncooked samples, protein structures did not break in the maximum travel of the plunger (10 mm), with no differences observed between samples at 5 mm penetration, the situation in heated samples is well-distinct. Gel network formation is apparent (LSH and HSH samples), and the plunger penetration results in a point of gel rupture, but this is only visible at penetration values higher than 5 mm (results not shown).

Texture Profile Analysis. Texture profile analysis (**Table 2**) showed that Hd, Sp, and Cw were highest ($P < 0.05$) in the high salt (HSH) samples and lowest ($P < 0.05$) in the control samples (no salt). A positive significant correlation between Hd ($R = 0.983$, $P < 0.0005$), Cw ($R = 0.982$, $P < 0.0005$), and salt content was observed. The higher textural properties (**Tables 1** and **2**) of the HSH sample were to be expected, since NaCl solubilizes meat proteins, and this increases the number of locations in the polypeptide chains able to interact during heating. The result is a stable, elastic, and rigid protein gel matrix with better water binding properties (*34*).

Table 2. Texture Profile Analysis of Heated Meat Batters[a]

sample	hardness (N)	springiness (dimensionless)	cohesiveness (mm)	chewiness (N × mm)
CH	34.2 ± 0.4 c	7.71 ± 0.05 b	0.68 ± 0.02 a	178.7 ± 2.9 c
LSH	38.3 ± 0.4 b	7.95 ± 0.04 a	0.71 ± 0.01 a	216.2 ± 2.8 b
HSH	41.4 ± 0.3 a	8.11 ± 0.14 a	0.73 ± 0.01 a	244.4 ± 2.9 a

[a] CH, heated control meat batter; LSH, heated low salt (1%) meat batter; HSH, heated high salt (2.5%) meat batter. Different letters (a, b, c) in the same column indicate significant differences ($P < 0.05$).

Raman Spectroscopy Analysis. Raman spectra of the control and high salt content of unheated and heated samples in the 950−1750 cm^{-1} region are shown in **Figure 1**. The assignments of the Raman bands have been carried out according to literature references (*16, 27*). The frequency and intensity changes in the Raman bands were the main indicatives of changes in the secondary protein structure and of variations in the local environments of meat proteins.

Protein Secondary Structure. The Raman band centered near 1656 cm^{-1} (**Figure 1**) was assigned to the amide I vibrational mode, which involves mainly C═O stretching and to a lesser extent N−H in-plane bending of peptide groups (*16, 21, 22*). The strongest intensity of the 1656 cm^{-1} band in the Raman spectra of these meat batters (**Figure 1**) can be attributable to proteins with high α-helix content (*16, 21, 22, 32*). A comparison of amide I band of control and high salt meat batters, unheated and heated, showed a frequency upshifting (1660 cm^{-1}), which indicated a decrease in α-helical structure resulting from the heating process. However, no changes in the frequency of amide I band were identified after adding salt in both unheated and heated samples (**Figure 1**).

Analysis of amide I spectra profile (*21, 22, 32*) provided a quantitative estimation of the percentages of secondary structure for the different meat batters (**Table 3**). There was a significant decrease ($P < 0.05$) in α-helix accompanied by a significant increase ($P < 0.05$) in β-sheet structure after heating (**Table 3**), which are consistent with the amide I spectra changes (**Figure 1**). A negative correlation between α-helical structure ($R = -0.974$, $P < 0.0005$) and heating process and a positive correlation between β-sheet ($R = 0.961$, $P < 0.0005$) versus heating were found. The results also showed an intensity decrease trend in the Raman band assigned to the C−C stretching vibration (940 cm^{-1}) as a function of thermal treatment (**Table 4**). This Raman spectral change is correlated with a gradual loss of α-helical structure (*16, 21, 22*), which confirms the secondary structure results obtained from the amide I spectra profile analysis (**Table 3**). In agreement with these spectroscopic findings, a decrease in α-helical structure has been shown by Raman spectroscopy in surimi cooked gels (*25, 26, 35*). Studies of heat-induced structural changes in meat myofibrillar proteins, determined by FT-IR spectroscopy, have also indicated an increase in β-sheet structure levels (*11–15, 36*). Previous differential scanning calorimetry and electrophoresis works also indicated that thermal treatment (70 °C) of meat batter produce almost complete protein denaturation and aggregation, mostly through hydrophobic interactions (*9, 10*). It is reasonable to assume that the establishment of a stable gel network upon heating is closely linked to a hydrogen-bonded β-sheet structure increasing. Studies in roasted beef indicated that the α-helical

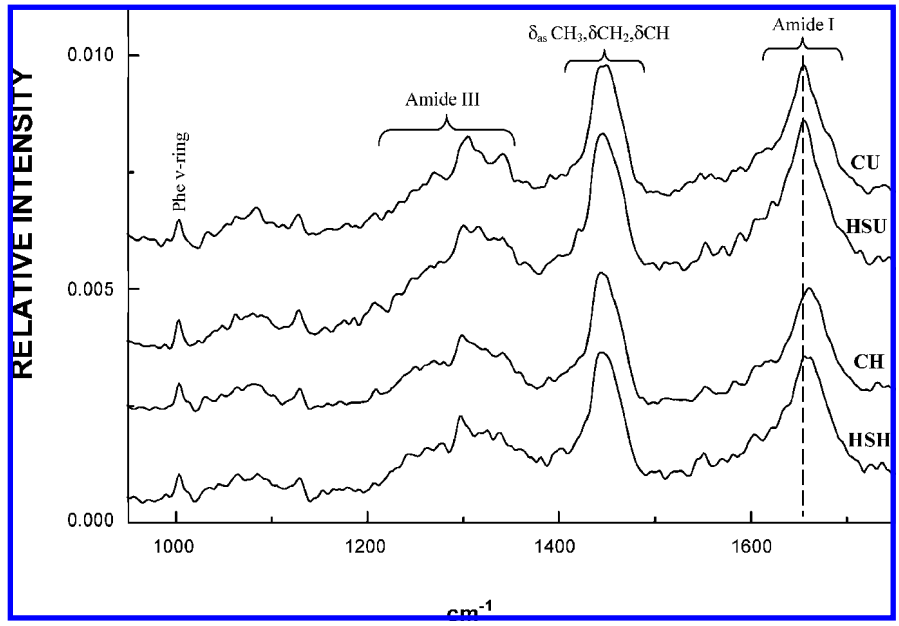

Figure 1. Raman spectra in the 950−1750 cm^{-1} region from different meat batter analyzed: CU, unheated control meat batter; HSU, unheated high salt (2.5%) meat batter; CH, heated control meat batter; HSH, heated high salt (2.5%) meat batter.

Table 3. Percentages of Protein Secondary Structure (α-Helix, β-Sheet, Turns, and Unordered) of the Unheated and Heated Meat Batters[a]

sample	α-helix	β-sheet	turn	unordered
CU	59.2 ± 0.9 a,x	15.7 ± 1.7 a,y	14.6 ± 0.7 a,x	10.6 ± 0.6 a,x
LSU	59.3 ± 0.8 a,x	16.6 ± 1.5 a,y	14.3 ± 0.3 a,x	9.9 ± 0.4 a,x
HSU	60.7 ± 1.7 a,x	15.3 ± 1.1 a,y	14.4 ± 1.2 a,x	9.6 ± 0.8 a,x
CH	48.5 ± 1.1 a,y	24.5 ± 1.0 a,x	16.1 ± 0.8 a,x	10.9 ± 0.7 a,x
LSH	47.8 ± 0.9 a,y	26.3 ± 1.5 a,x	15.1 ± 1.2 a,x	10.8 ± 0.6 a,x
HSH	46.8 ± 1.2 a,y	26.9 ± 1.9 a,x	15.3 ± 0.9 a,x	10.9 ± 0.4 a,x

[a] Samples description: CU, unheated control meat batter; LSU, unheated low salt (1%) meat batter; HSU, unheated high salt (2.5%) meat batter; CH, heated control meat batter; LSH, heated low salt (1%) meat batter; HSH, heated high salt (2.5%) meat batter. Different letter (a, b, c) in the same treatment, unheated (CU, LSU, HSU) or heated (CH, LSH, HSH) indicate significant differences ($P < 0.05$). Different letters (x, y, z) in the same sample unheated and heated indicate significant differences ($P < 0.05$).

Table 4. Normalized Intensities of the Tryptophan Band (759 cm^{-1}), Tyrosyl Doublet (850/830 cm^{-1}), νCC Band (940 cm^{-1}), and νCH Band (2935 cm^{-1}) of the Unheated and Heated Meat Batters[a]

sample	Trp band (I_{759}/I_{1003} cm^{-1})	Tyr doublet (I_{850}/I_{830} cm^{-1})	νCC band (I_{940}/I_{1003} cm^{-1})	νCH band (I_{2935}/I_{1003} cm^{-1})
CU	0.28 ± 0.05 a,x	0.87 ± 0.11 a,x	0.53 ± 0.12 a,x	27.1 ± 1.2 a,x
LSU	0.28 ± 0.04 a,x	0.76 ± 0.22 a,x	0.51 ± 0.14 a,x	28.2 ± 1.1 a,x
HSU	0.30 ± 0.02 a,x	1.07 ± 0.16 a,x	0.49 ± 0.10 a,x	30.2 ± 1.5 b,x
CH	0.28 ± 0.06 a,x	0.85 ± 0.11 a,x	0.38 ± 0.10 a,x	25.1 ± 0.9 a,y
LSH	0.29 ± 0.06 a,x	0.93 ± 0.12 a,x	0.32 ± 0.15 a,x	26.3 ± 1.8 a,x
HSH	0.29 ± 0.05 a,x	1.01 ± 0.21 a,x	0.39 ± 0.11 a,x	27.0 ± 1.7 a,x

[a] Samples description: CU, unheated control meat batter; LSU, unheated low salt (1%) meat batter; HSU, unheated high salt (2.5%) meat batter; CH, heated control meat batter; LSH, heated low salt (1%) meat batter; HSH, heated high salt (2.5%) meat batter. Different letter (a, b, c) in the same treatment, unheated (CU, LSU, HSU) or heated (CH, LSH, HSH) indicate significant differences ($P < 0.05$). Different letters (x, y, z) in the same sample unheated and heated indicate significant differences ($P < 0.05$).

to β-sheet ratio is an important factor contributing to the shear force, tenderness, acceptability of texture, and overall acceptability of meat (*19*). In these conditions, a higher proportion of a β-sheet structure was correlated with tough meat (*19*). In this connection, in the present work, the penetration force values of heated samples were significantly ($P < 0.05$) higher than in unheated samples, which could be linked with the highest β-sheet structure upon thermal treatment (PF control unheated = 0.19 ± 0.03 N; PF control heated = 3.59 ± 0.29 N). A positive significant correlation ($R = 0.968$, $P < 0.0005$) between β-sheet structure content and penetration force values and a negative significant correlation ($R = -0.974$, $P < 0.0005$) between α-helix and penetration force were found.

Results of secondary structure showed that there were not significant differences as a function of salt content in both unheated and heated samples (**Table 3**). However, a trend to increase the β-sheet content as a function of the presence of salt in heated samples could be observed. In addition, a positive significant correlation between β-sheet structure and Hd ($R = 0.736$, $P < 0.05$), Ch ($R = 0.750$, $P < 0.05$), Cw ($R = 0.735$, $P < 0.05$), and NaCl added ($R = 0.735$, $P < 0.05$) was found in heated samples. Some authors have reported changes in protein secondary structure upon addition of inorganic salts in isolated myosin solutions (*24*). The stronger secondary structural changes observed in myosin in the presence of inorganic salts such as $CaCl_2$, MgCl, and LiBr can be explained by considering that the contact surface in the case of myosin solutions is much greater than in the myofibrillar systems studied here. FT-IR spectroscopic analysis of pork muscle, subjected to brine salting at different concentrations, has shown protein structural changes in terms of decreases in α-helix content (*13, 14*) at salt concentrations higher than 3%. It is reasonable to assume that salt addition in heated meat batter could produce an increase in β-sheet structure, which could be correlated with the enhancement of textural properties found in heated samples upon NaCl addition (**Table 3**). Our results also showed that there is a significant ($P < 0.05$) increase in water binding properties of heated samples when the salt content increases (**Table 1**). The specific myofibrillar protein structures could be decisive for the myofibrillar protein network ability to trap water and the characteristics of the intramyofibrillar water population. Therefore, the β-sheet structure tendency to increase upon thermal treatment as a function of NaCl concentration could be related to the water binding properties (**Tables 2** and **3**). A positive

correlation ($R = 0.683$, $P < 0.05$) between water binding properties and β-sheet structure content was found in heated samples.

Amide III band is another Raman band that provides information about secondary structure of proteins (*16, 21, 22*). This spectroscopic Raman band involves C−N stretching and N−H in-plane bending vibrations of the peptide bond as well as contributions from Cα−C stretching and C═O in-plane bending. The amide III band is difficult to interpret because vibrational spectroscopy of proteins produces a complex pattern of bands in the 1225−1350 cm^{-1} range (**Figure 1**). However, a slight increasing intensity can be observed in the range 1225−1240 cm^{-1} range upon thermal treatment of meat batters (**Figure 1**). This spectral change can be attributable to β-sheet formation (*16, 21, 22*), which is consistent with the percentages of protein secondary structure obtained from amide I profile analysis (**Table 3**).

Tryptophan Residues and Tyrosine Doublet Bands. The changes in these Raman bands can mainly provide information about hydrophobic interactions of proteins. In this way, the band assigned to tryptophan residues near 759 cm^{-1} indicates changes in the local environment of the tryptophan aromatic moiety. It has been reported that a decrease in the intensity of this Raman band is attributable to solvent exposure of Trp residues in proteins (*16, 21, 22*). However, in the present work, no significant ($P > 0.05$) differences were found in the intensity of this Trp band (**Table 4**) upon thermal treatment and/or salt content. The tyrosyl doublet ratio (I_{850}/I_{830}) has been proposed to determine whether the tyrosine residue is solvent exposed or buried (*16, 21, 22*). **Table 4** showed that the values of the tyrosyl doublet ratio (I_{850}/I_{830}) ranged between 0.8 and 1.1; therefore, the tyrosine residues are buried in a hydrophobic environment independently of thermal treatment and NaCl content. Raman spectroscopy studies in surimi gels have showed an increase of tyrosyl doublet ratio as an effect of cooking process (*25*). However, in the present work, no significant differences ($P > 0.05$) in the tyrosil doublet ratio were found as a result of thermal treatment and salt addition (**Table 4**).

Aliphatic C−H Stretching Vibrations. Hydrophobic groups of proteins exhibit C−H stretching vibrational bands in the 2800−3000 cm^{-1} region (*16, 21, 22*). The Raman band at 2935 cm^{-1} is assigned to $\nu_{as}CH_2$ and ν_sCH_3 stretching vibrations (**Table 4**). It was reported that protein unfolding leading to solvent exposure of methyl and methylene groups produces spectral changes of the 2935 cm^{-1} band in terms of frequency upshifting and/or intensity increasing (*25*). In this respect, no shifts of the 2935 cm^{-1} band were observed (results not shown). However, in unheated samples, it has been noticed that there is an increase ($P < 0.05$) in the intensity of this band as a result of 2.5% salt addition (**Table 4**), which is indicative of exposure of hydrophobic aliphatic residues (*16, 21, 22*). These results could be interpreted in the sense that the addition of NaCl to meat batter causes structural changes in meat proteins involving dissociation of hydrophobic contacts between protein chains (*37*). By contrast, thermal treatment of control samples produced a decrease ($P < 0.05$) in the 2935 cm^{-1} band (**Table 4**), which could be attributed to an increase in the hydrophobic interactions of aliphatic residues (*16, 21, 22*). Similarly, a slight decrease in the intensity of the Raman band assigned to C−H stretching vibration of aliphatic residues was observed after cooking of surimi (*25*). In addition, only a trend toward decreasing intensity of the 2935 cm^{-1} band in salt containing meat batter was observed upon heating (**Table 4**). On the other hand, significant increasing of β-sheet structure in these samples upon heating could be observed (**Table 3**). It is well-known that β-sheet structure comprises characteristic hydrogen bonds involving the polypeptide backbone. Therefore, it is reasonable to assume that thermal treatment of meat batters containing salt involves mainly rearrangement of hydrogen bonds resulting in β-sheet formation. These results are consistent with selective soluble protein studies showing that the molecular associations involved in gel network formation during the elaboration of meat products, such as frankfurter, comprise β-sheet hydrogen-bonding and hydrophobic interactions, which are equally involved in protein denaturation and aggregation (*9*).

This investigation shows that Raman spectroscopy is a useful technique to evaluate the protein structural changes that occur upon thermal treatment and/or salt addition. Thermal treatment results in changes in the textural properties and the secondary and tertiary structure of meat protein. Decreases in α-helix structure accompanied by increases in β-sheets and hydrophobic interactions were observed upon heating. These protein structural changes could be related to an enhancement of textural properties in terms of increase of penetration force, hardness, and chewiness. Therefore, it is reasonable to assume that the establishment of a stable gel network upon heating is closely related to a β-sheet structure increasing. Increase of salt content in heated meat batter produces an increase in β-sheet structure, which could be related to change of texture (increases in hardness and chewiness) and improves water binding properties. The relationship between changes in the protein structure and the modifications in water binding and textural properties of meat batter open new possibilities that could be used to optimize the development of meat products when thermal treatment and/or salt addition are used. Structural studies concerning the reduction of salt in meat products could offer scientific and technological knowledge, which could help to improve the development of healthier meat products.

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