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Raman spectroscopy study of the structural effect of microbial transglutaminase on meat systems and its relationship with textural characteristics

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Abstract

Raman spectroscopy and texture analysis (TPA) studies were carried out to determine the effect of adding different levels of microbial transglutaminase (MTGase) to meat systems. This addition produced a significant ($p < 0.05$) increase in hardness, springiness and cohesiveness in the meat systems. Raman spectroscopy analysis revealed the occurrence of secondary structural changes in meat proteins due to MTGase. Modifications in the amide I (1650–1680 cm^{-1}) and amide III (1200–1300 cm^{-1}) regions indicated a significant ($p < 0.05$) decrease in α-helix content, accompanied by a significant ($p < 0.05$) increase in β-sheets and turns due to the addition of the enzyme to meat systems. Significant ($p < 0.05$) correlations were found between these secondary structural changes in meat proteins and the textural properties (hardness, adhesiveness, springiness and cohesiveness) of meat systems.

Keywords: Meat systems; Microbial transglutaminase; Texture profile analysis; Raman spectroscopy

1. Introduction

Over the past few years, the use of cold-set binders is growing for the manufacture of meat products, because of the numerous advantages they offer for the meat industry and consumer alike. Different commercial cold-set binding systems (alginate-calcium, transglutaminases of different origin, fibrinogen and thrombin) are commercially available for meat product manufacture. They can be used in the refrigerated and raw state without detrimental changes to the sensory characteristics of the final product (Cambero, López, de la Hoz, & Ordoñez, 1991; Kuraishi, Yamazaki, & Yasuyuki, 2001; Motoki & Seguro, 1998; Yokoyama, Nio, & Kikuchi, 2004). The use of these cold-set binders in meat product formulation also permits the food to be manufactured without or with reduced content of, phosphate and salt, which are traditionally related with an increased risk of bone and cardiovascular diseases (Kuraishi et al., 2001). A cold-set binding agent which has given good results for meat product formulation is the enzyme transglutaminase (TGase; protein-glutamine γ-glutamyltransferase, EC 2.3.2.13). This enzyme catalyses an acyl transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors), including the ε-amino group of lysine residues in certain proteins (Kuraishi et al., 2001; Motoki & Seguro, 1998; Yokoyama et al., 2004). The formation of ε-(γ-glutamyl)lysine isopeptide bonds results in both intra- and intermolecular cross-linking of proteins, leading to polymerisation (Kuraishi et al., 2001; Motoki & Seguro, 1998; Totosaus, Montejano, Salazar, & Guerrero, 2002; Yokoyama et al., 2004). Some authors have studied in model systems (protein isolates),

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by SDS–PAGE or size exclusion chromatography the formation of these polymerised proteins and their functional properties (Aktas & Kilic, 2005; De Jong & Koppelman 2002; Liu & Damodaran, 1999). Moreover, differential scanning calorimetry (DSC) has been used to determine protein structural changes due to transglutaminase in protein isolates (Aktas & Kilic, 2005; Ramirez-Suarez & Xiong, 2002; Siu, Ma, & Mine, 2002).

The production of transglutaminase for industrial use was made possible by the isolation and purification of a bacterial TGase from a microorganism taxonomically classified as a variant of *Streptoverticilium mobaraense* (Kuraishi et al., 2001; Motoki & Seguro, 1998; Yokoyama et al., 2004). It has been shown that microbial transglutamianse improves functional and textural properties of many food proteins, such as myosin. There have been numerous studies about quality characteristics, such as functional and textural properties of meat products manufactured with transglutaminase (Carballo, Ayo, & Jiménez-Colmenero, 2006; Kilic, 2003; Kuraishi et al., 2001; Pietrasik & Li-Chan, 2002). It has been suggested that the use of transglutaminase in the manufacture of meat products can result in the introduction of substantial changes in the structure of meat proteins, producing functional and textural modifications. However, studies of the structural changes produced in proteins by transglutaminase have only been carried out in model systems (protein isolates). To better control the changes in meat products caused by transglutaminase, the enzymatic polymerisation process should be monitored directly, without pre-treating the sample. Structural information relating to aspects of how the microbial transglutaminase affects the structure of the finished meat product is necessary, to optimise the conditions of enzyme application.

Raman spectroscopy is a non-invasive and direct technique which provides structural information on the secondary and tertiary structure of proteins (Herrero, In Press; Li-Chan, Nakai, & Hirotsuka, 1994) and has proven to be a powerful technique for investigating the structure of water and lipids (Beattie, Bell, Borgaard, Fearon, & Moss, 2006; Herrero, Carmona, García, Solas, & Careche, 2005; Li-Chan, 1996; Olsen, Rukke, Flatten, & Isaksson, 2007). Raman spectroscopy has been shown to be a useful tool to study protein structure in solid food systems, such as meat (Herrero, In Press), and, in turn, to study the structure of isolated meat proteins, such as myosin (Carew, Asher, & Stanley, 1975; Carew, Stanley, Seidel, & Gergely 1983). Several studies have compared Raman spectroscopy to traditional methods, to determine modifications in meat lipids (Beattie et al., 2006; Olsen et al., 2007). The possibility has also been studied of using protein structural changes determined by Raman spectroscopy to predict protein functional properties and sensory attributes of meat (Beattie, Bell, Farmer, Moss, & Patterson, 2004; Brøndum, Byrne, Bak, Bertelsen, & Engelsen, 2000; Pedersen, Morel, Andersen, & Engelsen, 2003). However, to our knowledge, no Raman spectroscopic study has been carried out, to examine the changes in structural properties of transglutaminase-treated meat products.

The aim of this work was to study by Raman spectroscopy structural changes in meat systems produced by microbial transglutaminase. These structural changes have also been related to changes in the textural properties of the meat systems.

2. Materials and methods

2.1. Experimental design

Fresh pork (*M. biceps femoris*) was purchased from a local abattoir at 48 h *post mortem*. ACTIVA EB, binding effect, (Ajinomoto Co., Inc., Tokyo, Japan) was obtained as a free sample from Impex Química S.A. (distributor in Spain). ACTIVA EB, used in freeze-dried form, was composed of sodium caseinate (60%), maltodextrin (39.5%) and microbial transglutaminase (MTGase) at 0.5%, as enzyme with the ability to cross-link proteins.

Three different types of meat systems were considered with different levels of MTGase: (1) meat emulsion with MTGase at 0% (ME00), (2) meat emulsion with MTGase at 0.05% (MET05) and (3) meat emulsion with MTGase at 0.10% (MET10).

2.2. Preparation of meat systems

The visible fat and connective tissue was removed from the meat. Then, meat was added to a cutter (Robot Coupe R 8 V.V., Vincennes Cedex, France) where the material was cut and, after that, was mixed with water to obtain a homogeneous mixture. The process was performed while controlling the temperature of the product at 0–2 °C. With this procedure three different meat systems with 0%, 0.05% and 0.10% of MTGase were prepared: (1) 90 g of pork was mixed with 10 ml of water (ME00), (2) 80 g of pork was mixed with 10 ml of water and 10 g of ACTIVA EB (MET05), and (3) 70 g of pork was mixed with 10 ml of water and 20 g of ACTIVA EB (MET10). These mixtures were gently prepared in a mixer adding the ingredients in the following order: meat emulsion, water and ACTIVA EB.

The meat systems (ME00, MET05 and MET10) were extended (1 cm thickness) in circular plastic containers (9 cm diameter) and immediately afterwards were individually vacuum-packaged (up to 20 kPa) into plastic bags (CRYOVAC) in a Vapta 750/400 model machine (Vapta SL, Madrid, Spain). They were then stored for 72 h at 4 °C until analysis. This procedure was performed in quadruplicate.

In addition, to check the possible effect of sodium caseinate and maltodextrin (non-enzyme components of ACTIVA EB) on the meat system models, two mixture controls were prepared: (a) pork, water, sodium caseinate and maltodextrin (80/10/6/4, w/v/w/w) and (b) pork, water, sodium caseinate and maltodextrin (70/10/12/8, w/v/w/w).

2.3. Chemical analysis

The protein (Kjeldahl nitrogen) and fat content of meat used to manufacture the different meat systems were determined using the methods of AOAC (1995), and Bligh and Dyer, described by Hanson and Olley (1963), respectively.

2.4. Textural analysis

Texture profile analysis (TPA) of different meat systems was performed at around 22 °C using a TA.XT2i SMS Stable Microsystems Texture Analyser (Stable Microsystems Ltd., Godalming, Surrey, England) with the Texture Expert program. This procedure involved the preparation of four cylinders of 1 cm height and 2 cm width from each sample. A double compression cycle test was performed up to 50% compression of the original portion height with an aluminium cylinder probe P/25. A time of 5 s was allowed to elapse between the two compression cycles. Force–time deformation curves were obtained with a 25 kg load cell applied at a cross-head speed of 2 mm/s. The following parameters were quantified (Bourne, 1978): hardness (N), the maximum force required to compress the sample, springiness (m), the ability of the sample to recover its original form after the deforming force was removed; adhesiveness ($N \times s$), the area under the abscissa after the first compression; and cohesiveness, the extent to which the sample could be deformed prior to rupture. Four replicates were performed for each type of sample.

2.5. Raman spectroscopy analysis

Portions of meat systems with or without MTGase active ingredient were transferred to glass tubes (5 cm height and 5 mm i.d.; Wilmad Glass Co., Inc., Buena, NJ) to fill to approximately 1cm depth (Herrero et al., 2005). 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 $\pm 0.5\ cm^{-1}$, 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. Two thousand scans (about 60 min) were recorded for each sample, which was analysed in triplicate, resulting in a total of 6000 scans per sample. Measurements were performed on four samples for each meat system. Raman spectra were processed using Opus 2.2 (Bruker, Karlsruhe, Germany) and Grams/AI (Thermo Electron Corporation, Waltham, MA) software. The Raman spectra of all the samples were recorded in the solid state.

The Phe ν-ring band located near 1003 cm^{-1} was used as internal standard to normalise the spectra, as it has been reported to be insensitive to the micro-environment (Herrero, In Press; Li-Chan, 1996). 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 (Li-Chan et al., 1994). The intensity values of Raman bands from various atomic groups were determined after spectral normalisation. Protein secondary structures were determined as percentages of α, β, turns and random coil or unordered conformations (Alix, Pedanou, & Berjot, 1988). With this aim, the water spectrum was previously subtracted from the spectra by following the same criteria as those described in other literature (Alix et al., 1988). Analysis of the Raman spectra of the meat system models (MET00, MET05 and MET10), the mixture controls (combinations of different levels of meat and sodium caseinate and maltodextrin) and ACTIVA EB showed that the proportions of the components of this commercial product did not interfere with the spectral profile, in terms of secondary (amide I and III bands) and tertiary (tryptophan residues, tyrosil doublet ratio, 1450 cm^{-1} and 2930 cm^{-1} bands) structure. In this way, it is reasonable to assume that the structural changes observed in the meat system models can be attributed to the MTGase effect.

2.6. Statistical analysis

An individual meat system was the experimental unit for all data analysis. To check the normal distribution (90% confidence) of samples, the Shapiro–Wilks test was applied. When samples fitted the normal distribution, one-way ANOVA analysis was performed. When samples did not fit the normal distribution, the Kruskal–Wallis test was used to test the null hypothesis that the medians of variable within each of the levels of samples were the same. Duncań s test for multiple mean comparisons, Pearson product moment correlation (r) and multiple regression analysis (using a Durbin-Watson statistical test, at 95% of confidence level) were performed to determine the relationships between data obtained by TPA and Raman spectroscopy analysis. Statistical analysis was carried out using Statgraphics Plus version 5.0 (StatPoint Inc., Herndon, VA). Data were presented as the means and standard deviations (SD) of each model system.

3. Results and discussion

3.1. Chemical and textural properties

The meat emulsions used to manufacture the system models had similar chemical compositions, with percentages of protein and fat of about 19.0 ± 1.1 and 5.1 ± 0.3, respectively.

Results of the texture profile analysis (TPA) are shown in Fig. 1. There were significant ($p < 0.05$) differences in TPA parameters between systems with and without

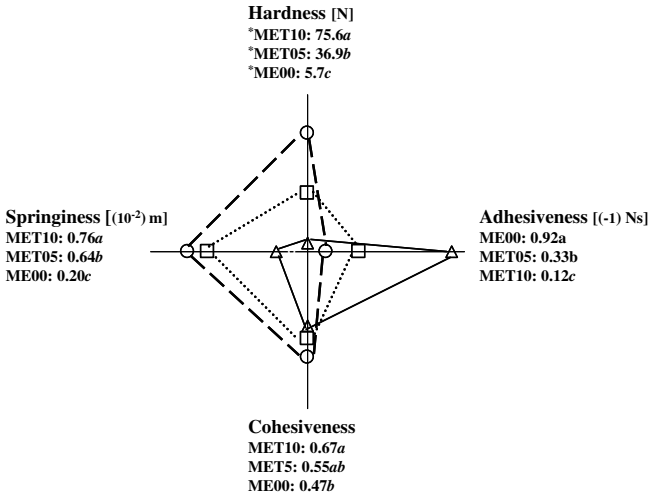

Fig. 1. Textural profile analysis (TPA) parameters of the different types of meat systems analyzed: meat emulsion (ME00, Δ, –), meat emulsion with MTGase at 0.05% (MET05, □,···) and 0.10% (MET10, ○, - - -). *Mean values with different letters (a, b, c) for the same texture parameter indicate significant differences ($p < 0.05$).

MTGase. The highest ($p < 0.05$) value of adhesiveness corresponded to the ME00 system and there was a significant negative correlation between this parameter ($r = -0.989$, $p < 0.01$) and MTGase concentration (Fig. 1). TPA results also indicated that hardness, springiness and cohesiveness were higher ($p < 0.05$) in meat systems supplemented with MTGase (MET05 and MET10) than in the ME00 system (no enzyme addition). Similar results were reported by other authors, who indicated that transglutaminase has the potential to increase firmness and springiness, among other properties (Carballo et al., 2006; Kuraishi et al. 2001). Our results showed a significant positive correlation between hardness ($r = 0.994$, $p < 0.005$) versus enzyme addition. The increase ($p < 0.05$) in hardness, springiness and cohesiveness observed in meat systems with MTGase could be explained by the transglutaminase enhancing protein cross-linking. This results in the formation of large polymeric protein molecules and a gel structure between the meat particles, and also cross-linking of this gel with proteins at the meat surface (De Jong & Koppelman, 2002; Totosaus et al., 2002). The high ($p < 0.05$) adhesiveness and low hardness, cohesiveness and springiness of the MET00 system model indicated a pseudoplastic fluid behaviour because of a lack of myofibrillar protein gelation. In this respect, transglutaminase has been proposed as a useful tool to improve the functional and textural properties of food (Kuraishi et al., 2001; Motoki & Seguro, 1998; Yokoyama et al., 2004).

The mixture controls elaborated with combinations of pork, water, sodium caseinate, maltodextrin, without MTGase, showed TPA parameters (data not shown) close to that of the MET00 model (meat emulsion and water). These results could indicate that the textural profile changes observed in models supplemented with MTGase (MET05 and MET10) are not due to the non-enzyme components of ACTIVA EB.

3.2. Raman spectroscopic analysis

Raman spectra of the different meat systems (ME00, MET05 and MET10) in the 700–1800 cm^{-1} region are shown in Fig. 2. The assignments of the corresponding bands are included in Table 1. The frequency and intensity changes in the Raman bands were mainly indicative of changes in the secondary structure and variations in local environments of meat proteins.

3.2.1. Amide I and amide III bands

The Raman band centred near 1653 cm^{-1} (Table 1), was assigned to the amide I vibrational mode (Herrero, In Press; Li-Chan et al., 1994), which involves mainly C=O stretching and, to a lesser extent, C–N stretching, C_α–C–N bending, and N–H in-plane bending of peptide groups. There has been shown previously to be a correlation between the frequencies of the amide I band components and the types of protein backbone conformation (Alix et al., 1988; Herrero, In Press; Li-Chan et al., 1994). Generally speaking, proteins with a high α-helical content show an amide I band centred around 1650–1658 cm^{-1}, while those with β-sheet structures show an intensity maximum in the 1665–1680 cm^{-1} range. On the other hand, high proportions of random coil or unordered structure are attributable to proteins with an amide I band centred at 1660–1665 cm^{-1}. In this respect, the strongest intensity of the 1653 cm^{-1} band in the spectra of meat systems can be attributable to proteins with a high α-helix content (Fig. 2). The intensity maximum of this band can be observed to shift to higher frequencies with increasing amounts of MTGase (MET05 and MET10) (Fig. 2). A reasonable explanation for this spectral change may be a

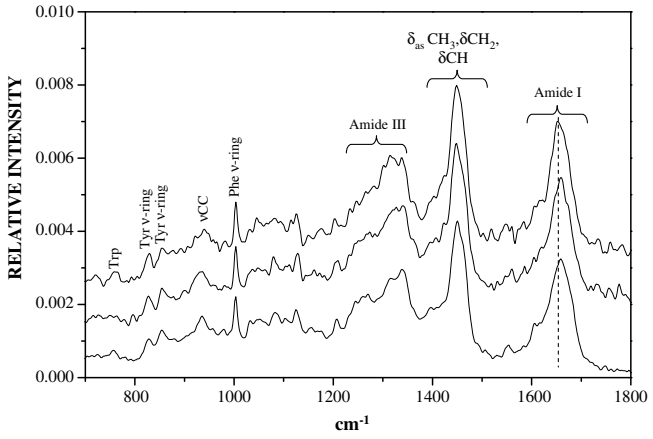

Fig. 2. Raman spectra in the 700–1800 cm^{-1} region from the different types of meat systems analysed: meat emulsion (ME00) (upper), meat emulsion with MTGase at 0.05% (MET05) (middle) and 0.10% (MET10) (lower).

Table 1
Assignment of some Raman bands of meat emulsion systems

Frequency (cm^{-1})	Assignment
620–640 w	Phe
644 w	Tyr
760 m	Trp
830 w	Tyr ν-ring
850 w	Tyr ν-ring
880 w	Trp ν-ring
940 m	νCC (α-helix)
1003 m	Phe ν-ring
1250 sh	Amide III (β-helix)
1273 m	Amide III (α-helix)
1309 sh	Amide III (α-helix)
1321 m	δ CH
1341 m	δ CH
1360 w	Trp ν-ring
1400–1430 w	$\nu_s COO^-$ (Asp, Glu)
1425 sh	Asp, Glu, Lys
1450 s	$\delta_{as}CH_3$, δCH_2, δCH
1554 w	Trp ν-ring
1645–1685 vs	Amide I
2935 vs	CH_3, νCH_2, νCH

Abbreviations: vs, very strong; s, strong; m, medium; w, weak; sh, shoulder; ν, stretching; δ, bending.

decrease in α-helical structure resulting from enzyme addition.

Quantitative information about the secondary structure of proteins can be obtained from the amide I spectral profile (Alix et al., 1988; Herrero, In Press). Fig. 3 shows the structural profiles and percentages of secondary structure calculated from the amide I spectral profile for the different meat systems analysed (ME00, MET05 and MET10). There were significant ($p < 0.05$) differences in α-helical, β-sheet and turns structures between the various meat system studied. Meat systems with MTGase (MET05 and MET10) showed higher contents ($p < 0.05$) of β-sheet and turns than meat systems without transglutaminase (ME00). The highest values of turns content corresponded to the MET10 system (Fig. 3). A significant ($p < 0.05$) decrease in α-helix could be observed, accompanied by a significant ($p < 0.05$) increase in β-sheets and turns percentages upon addition of MTGase. Previously, an increase in the intensity of two intermolecular β-sheets bands has been shown by FT-infrared spectroscopy, associated with aggregate formation in oat globulin polymers formed by microbial transglutaminase (Siu et al., 2002). In the present work, no significant differences in unordered structure were found as a function of enzyme levels (Fig. 3). A negative correlation between α-helical structure ($r = -0.905$, $p < 0.01$) and the presence of MTGase, and a positive correlation between β-sheet ($r = 0.869$, $p < 0.02$) and turns conformations ($r = 0.989$, $p < 0.0002$) versus enzyme addition were found. These structural changes caused by MTGase addition could be attributed to the potential of MTGase to catalyse the covalent cross-linking of meat proteins (Kuraishi et al., 2001; Motoki & Seguro, 1998; Totosaus et al., 2002). MTGase can form ε-(γ-glutamyl) lysine bonds in meat proteins, and this cross-linking drastically alters the protein structure (Yokoyama et al., 2004). Some authors described the effects of MTGase on thermal and electrophoretic properties of isolated myofibrillar proteins of meat and indicated that MTGase catalysed polymer formation (Aktas & Kilic, 2005; Ramirez-Suarez & Xiong, 2002). Electrophoretic patterns of myofibrillar protein isolate incubated at 5 °C with 0.1% of transglutaminase showed the formation of high-molecular weight polypeptides (MW > 200 kDa), which could result from the ε-(γ-glutamyl)lysine isopeptide bonding (Ramirez-Suarez & Xiong, 2002). Results of differential scanning calorimetry analysis indicated that the modifications in thermal properties of myofibrillar proteins, caused by transglutaminase, were presumably due to structural changes in myosin, resulting from changes in the intramolecular bonds, as revealed by eletrophoretic patterns (Ramirez-Suarez & Xiong, 2002; Siu et al., 2002). Moreover, some authors indicated that changes in the thermal properties, caused by the addition of transglutaminase to myofibrillar proteins isolated from ground beef could be due to the formation of high weight molecular complexes with a compact and ordered conformation, which would have higher thermal stability than the unassociated proteins (Aktas & Kilic, 2005). The high-molecular weight polypeptides or aggregates formation enhanced by transglutaminase could be related to the secondary structural changes, in terms of an increase in β-sheet and turns structure observed by Raman spectroscopy in meat systems caused by addition of MTGase.

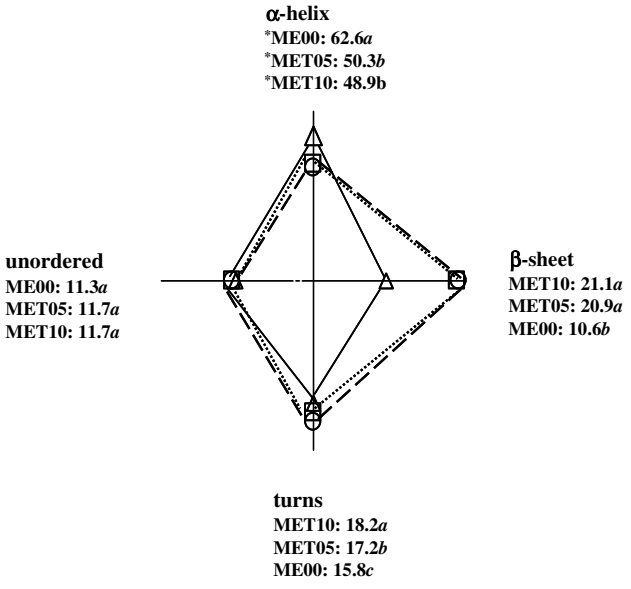

Fig. 3. Protein secondary structures (percentages) of: α-helix, β-sheet, turns and unordered, of the different types of meat systems analysed: meat emulsion (ME00, Δ, –), meat emulsion with MTGase at 0.05% (MET05, □,· · ·) and 0.10% (MET10, ○, - - -). *Mean values with different letters (a, b, c) for the same texture parameter indicate significant differences ($p < 0.05$).

The amide III band is another useful Raman band for providing information about the secondary structure of proteins (Herrero, In Press; Li-Chan et al., 1994). The amide III mode involves C–N stretching and N–H in-plane bending vibrations of the peptide bond, as well as contributions from C_α–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 (Table 1 and Fig. 2). The intensity of the amide III band for α-helix structure appears around 1300–1260 cm^{-1}, which overlaps with the region assigned for turns. Although the β-sheet and unordered structure bands overlap in the amide III region (1250 and 1240 cm^{-1}) an increasing intensity can be observed in the range 1225–1240 cm^{-1} in going from MET05 to MET10 systems (Fig. 2). This spectral change could be caused by β-sheet formation by enzyme addition, which is consistent with the quantitative estimates from amide I analysis (Fig. 3).

The changes in protein secondary structure observed in meat proteins caused by MTGase addition are accompanied by changes in the textural properties of meat systems. In this way, a positive significant correlation between adhesiveness and α-helical ($r = 0.961$; $p < 0.04$) and turns ($r = 0.987$; $p < 0.01$) structure, and a negative correlation with β-sheet content ($r = -0.934$; $p < 0.04$) was found. Springiness was correlated positively with β-sheet structure ($r = 0.966$; $p < 0.03$) and negatively with α-helix content ($r = -0.982$; $p < 0.02$). A positive correlation between hardness ($r = 0.961$; $p < 0.04$), springiness ($r = 0.960$; $p < 0.04$) and turns structure was also observed. Therefore, it is possible to assume that these secondary structural changes in the meat protein caused by MTGase addition are correlated with changes in the textural properties. These changes in protein secondary structure and textural properties could be attributed to the formation of high molecular weight complexes with a compact and ordered conformation of gel structure as a result of the addition of MTGase.

3.2.2. Local environments of meat proteins

Raman spectra of these samples show several bands which are characteristic of the tertiary structure of proteins (Fig. 2). The changes in these Raman bands could mainly provide information about hydrophobic interactions of proteins. In this way, the band assigned to tryptophan (Trp) residues near 758 cm^{-1} indicates changes to the aromatic amino acids. It has been indicated that a decrease in this Raman band is attributed to exposure of buried Trp residues in proteins (Herrero, In Press; Li-Chan et al., 1994). In the present work, no significant ($p > 0.05$) differences were found in the intensity of the Trp band at 759 cm^{-1} (Table 2). The tyrosyl doublet ratio (I_{850}/I_{830}) has been proposed to determine if the tyrosine (Tyr) residue is exposed or buried. When the intensity of the band at 850 cm^{-1} is higher than the intensity of the band near 830 cm^{-1} the Tyr residue is exposed. By contrast, when the I_{850} intensity is less than I_{830}, this could be interpreted as indicating an increase in Tyr residues buried within the protein network (Herrero, In Press; Li-Chan et al., 1994). Results showed that there is a trend towards a decrease in the Tyr doublet ratio (I_{850}/I_{830}), as a function of MTGase levels, although no significant ($p > 0.05$) changes were observed (Table 2). Some authors have used circular dichroism analysis to determine changes in the hydrophobic interactions of native MTGase due to modifications in Tyr and Trp residues. They concluded that high-pressure and high-thermal treatments are required to produce changes in Tyr and Trp residues of native MTGase (Menéndez, Rawel, Schwarzenbolz, & Henle, 2006).

Table 2
Normalised intensities of the 759 cm^{-1} (tryptophan) band, tyrosyl doublet at 855/828 cm^{-1} and 1450 cm^{-1} band ($\delta_{as}CH_3$, $\delta_{as}CH_2$, $\delta_{as}CH$) of the different types of meat systems analysed: meat emulsion (ME00), meat emulsion with MTGase at 0.05% (MET05) and 0.10% (MET10)

Normalised intensities	Systems		
	ME00	MET05	MET10
Trp band (I_{759}/I_{1003} cm^{-1})	0.20 ± 0.03a	0.17 ± 0.01ab	0.15 ± 0.02b
Tyr doublet (I_{855}/I_{828})	0.99 ± 0.05a	1.04 ± 0.04a	1.07 ± 0.05a
$\delta_{as}CH_3$, $\delta_{as}CH_2$, $\delta_{as}CH$ band (I_{1450}/I_{1003} cm^{-1})	3.42 ± 0.03a	3.04 ± 0.04b	2.91 ± 0.03c

a, b, c different letters in the same row indicate significant differences ($p < 0.05$).

The Raman bands at 1450 cm^{-1} and 2935 cm^{-1} are assigned to CH_2 and CH_3 bending and CH stretching vibrations respectively (Table 1). It has been indicated that the decreased intensity of these bands may result from hydrophobic interactions around the aliphatic residues (Herrero, In Press; Li-Chan, 1996; Li-Chan et al., 1994). Our results showed a significant ($p < 0.05$) tendency of the 1450 cm^{-1} band to decrease from ME00 to MET10 (Table 2). Concerning the 2935 cm^{-1} band, another significant ($p < 0.05$) decrease in normalised intensity was observed, in relation to increasing proportions of MTGase (mean, standard deviation and letters indicating significant differences of I_{2935}/I_{1003} values for ME = 27.9 ± 0.7a; MET05 = 26.8 ± 0.9a and MET10 = 24.6 ± 0.7b). Fig. 4 shows the intensity decrease of the 2935 cm^{-1} band with increasing enzyme level. These modifications could suggest that there is an increase in hydrophobic interactions in the meat systems with MTGase (MET05 and MET10). These results also suggest that the protein cross-linking could be largely due to this type of hydrophobic contact between aliphatic protein side chains.

3.3. Multiple linear regression analysis

The multiple linear regression analyses, using the TPA parameters as dependent variables and values from secondary structural changes (α-helical, β-sheet and turns structure) as independent variables, revealed a significant relationship between hardness ($r^2 = 0.954$, $p < 0.0001$), cohesiveness ($r^2 = 0.942$, $p < 0.0001$), adhesiveness ($r^2 =$

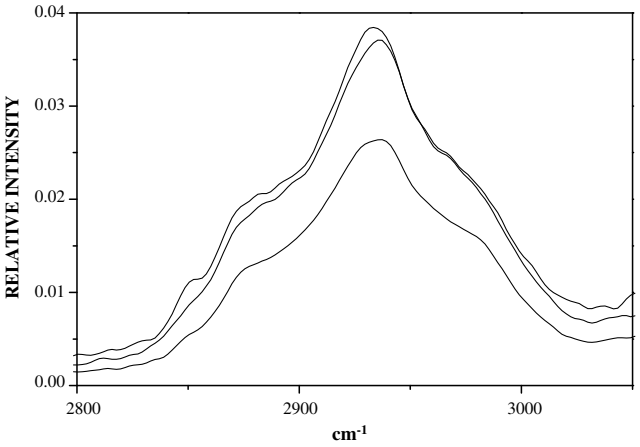

Fig. 4. Raman spectra in the 2800–3050 cm^{-1} from the different types of meat systems analysed: meat emulsion (ME00) (upper), meat emulsion with MTGase at 0.05% (MET05) (middle) and 0.10% (MET10) (lower).

0.978, $p < 0.0001$) and springiness ($r^2 = 0.985$, $p < 0.0001$) versus α-helix, β-sheets and turns content of the meat systems. The multiple linear regression models obtained in this study describe the following relationship between TPA and structural characteristics:

$$\text{Hardness} = 758.438 - 13.247 \times \alpha\text{-helix} - 14.434 \times \beta\text{-sheets} + 14.504 \times \text{turn}$$

The r^2 adjusted for degrees of freedom (a.d.f.) was 0.93 and the mean absolute error (m.a.e.) was 5.39.

$$\text{Cohesiveness} = 0.163 + 0.007 \times \alpha\text{-helix} + 0.023 \times \beta\text{-sheets} - 0.024 \times \text{turn}$$

(r^2 a.d.f = 0.92, m.a.e. = 0.01).

$$\text{Adhesiveness} = 21.139 - 0.279 \times \alpha\text{-helix} - 0.260 \times \beta\text{-sheets} - 0.116 \times \text{turn}$$

(r^2 a.d.f = 0.97, m.a.e. = 0.04).

$$\text{Springiness} = 7.121 - 0.096 \times \alpha\text{-helix} - 0.071 \times \beta\text{-sheets} - 0.009 \times \text{turn}$$

(r^2 a.d.f = 0.98, m.a.e. = 0. 03).

These preliminary results for the multivariate analysis confirm that some protein structural changes determined by Raman spectroscopy could be used to obtain regression models to predict TPA parameters of meat systems with added MTGase. Further research is required to obtain more data and robust mathematical models, in order to optimise the textural characteristics of meat products formulated with cold-set binding agent and especially with MTGase.

4. Conclusion

In conclusion, the addition of microbial transglutaminase (MTGase) notably modifies the textural properties of meat systems. This enzyme probably catalyses the cross-links between meat proteins, resulting in large polymeric protein molecules, with a gel structure. These processes of cross-linking and polymerisation profoundly modify the Raman spectra of meat protein. As a function of MTGase levels, increases in β-sheet and turns structure, accompanied by increases in hardness, springiness and cohesiveness and decreases in adhesiveness of meat systems were found. The relationship between changes in the protein structure and the modifications in meat system textural properties could be used to optimise meat product manufacture when transglutaminase is used.

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