Raman spectroscopic study of effect of the cooking temperature and time on meat proteins

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A B S T R A C T

Changes in protein secondary structure during thermal treatment of whole meat were studied using Raman spectroscopy. Pork longissimus thoracis was heat treated at 50 to 70 °C for 2–10 h and 110 average Raman spectra were collected from all the combinations. The effect of cooking temperature on meat protein structure was highly pronounced compared to cooking time. Detailed information about the changes in protein structure affected by cooking temperature and time was revealed and Raman spectra from different cooking temperatures were readily discriminated by principal component analysis. Temperature had a significant effect on the intensity ratio of tyrosine (Tyr) and tryptophan (Trp) and on cooking loss. Good correlations were found between the Raman spectra and cooking temperature (R² = 0.96), cooking loss (R² = 0.82) and cooking time (R² = 0.78). Raman spectroscopy proved to be a useful technique to follow the effect of cooking temperature and time on meat proteins in intact muscle.

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1. Introduction

Heat treatment of meat and meat products leads to structural changes in meat proteins. The structural changes in myofibrillar proteins, sarcoplasmic proteins and connective tissues will therefore have an effect on tenderness and/or texture and other quality parameters of a product. Some of the changes are transversal and longitudinal shrinkage of the muscle fibers, aggregation and gel formation of the sarcoplasmic proteins and shrinkage and solubilization of the connective tissues (Christensen, Bertram, Aaslyng, & Christensen, 2011; Christensen, Ertbjerg, Aaslyng, & Christensen, 2011; Frøst, Skibsted, & Risbo, 2012). Myofibrillar proteins, accounting for 50 to 55% of the total protein in muscle (Tornberg, 2005), are therefore governing constituents for the quality of heat treated products.

Cooking temperature and time are the main determinant factors which need attention when dealing with the heat treatment of meat. The effect of these two factors on the structure of meat proteins (Christensen, Bertram, et al., 2011; Christensen, Ertbjerg, et al., 2011) and in turn on product quality (Christensen, Bertram, et al., 2011; Christensen, Ertbjerg, et al., 2011; Mortensen, Frost, Skibsted, & Risbo, 2012) has been studied intensively. According to studies using differential scanning calorimetry (DSC), there are three maximum transition temperatures: 56.5, 65.8 and 79.4 °C which could be ascribed to myosin, sarcoplasmic proteins and connective tissues and actin in pork muscle, respectively. As mentioned above, conformational changes in myofibrillar proteins, sarcoplasmic proteins and connective tissues determine properties of the final product. It is, therefore, needed to look for fast and
non-destructive methods which can be used to monitor the final quality of a product.

Raman spectroscopy is a useful technique for monitoring changes in protein structure and provides information at the molecular level. It measures molecular vibrations but instead of measuring absorption such as in infrared (IR) spectroscopy, Raman spectroscopy measures scattering. The principle of Raman spectroscopy is that a sample is irradiated with a strong monochromatic laser light which interacts with a molecule in the sample. Most of the photons are scattered from the sample with the same energy level as the incident light (Rayleigh scattering) whereas very few photons are scattered with a lower frequency compared with the frequency of the incident light (Stokes scattering). The difference in frequency of the incident light and inelastically scattered light is called the Raman shift \( \Delta \nu \) in \( \text{cm}^{-1} \) or simply just the Raman spectrum. This spectrum contains information about the fundamental molecular vibrations as is also found in the IR spectrum. Near infrared (NIR) is another spectroscopic technique. It measures overttones and the combination tones of the molecular vibrations whereas IR and Raman spectroscopy measures the fundamentals. The main advantage of Raman spectroscopy over NIR is that Raman spectroscopy has a higher selectivity and that the spectra are not dominated by molecular vibrations of water which dominate the infrared and NIR spectra of many food samples (Ellekjær & Isaksson, 1992). In contrast, Raman spectroscopy has the disadvantage of being sensitive to sample fluorescence.

In the past few years, Raman spectroscopy has been used to predict the quality of meat and meat products (Herrero, Carmona, Cofrades, & Jimenez-Colmenero, 2008; Pedersen, Morel, Andersen, & Engelsen, 2003; Schmidt, Scheier, & Hopkins, 2013; Wang, Lonergan, & Yu, 2012). Beattie, Bell, Farmer, Moss, and Patterson (2004) found a good correlation between Raman spectra and consumer-perceived quality parameters of roasted beef. Another promising result has also been reported in beef. Wang et al. (2012) used Raman spectra to predict the sensory quality of pork loin (cooked at 70 °C) and found that prediction accuracy for tenderness and juiciness was significantly higher than that for chewiness. The authors (Wang et al., 2012) suggested that this apparent discrepancy in prediction accuracy could be due to the fact that Raman spectra of the meat may be more closely related to protein composition and/or structures of myofibrillar components than that of connective tissues.

The main objective of the present study was to investigate the effect of cooking temperature and time on the meat proteins’ structure in intact muscle using Raman spectroscopy. The second objective of this study was to predict cooking temperature and time using Raman spectra collected from cooked then cooled pork meat.

2. Material and methods

2.1. Meat sample

Four female pigs with a slaughter weight of 74.3 to 77 kg were stunned using CO₂ and further dressing was completed within 30 min post-mortem (PM) in a slaughter house (UCR, Roskilde, Denmark). The carcasses were chill-stored at 4 °C for 24 h PM. Longissimus thoracis was excised from both right and left sides of the pigs at the 24 h PM and was transported to the University of Copenhagen for laboratory analysis. The pH of the muscle was 5.52–5.58. Skin, back-fat and visible connective tissues were removed from the muscle. Sampling was made between the 9th and 13th ribs from both sides of the pigs and the sampled area was cut into chops (4–5 cm length). Each chop was vacuum packed separately and stored at −20 °C until use.

2.2. Heat treatment and sample preparation

The frozen chops were thawed at 4 °C for 15 h in their vacuum packed plastic bags. The thawed chops were removed from the plastic bags and cut into small pieces of samples (2 × 2 × 3 cm³, height × width × length, respectively). The length of the samples was cut along the direction of muscle fibers. Weight of each sample was recorded then each sample was re-vacuum packed and heat treated in a preheated water bath (ICC “Roner”, Frinox Aps, Hillerød, Denmark) at different cooking temperatures with an interval of 2 °C (50, 52, 54 … 68 and 70 °C) for different cooking times with an interval of 2 h (2, 4, 6, 8 and 10 h) in each cooking temperature. This design gave 55 treatments from all the combinations (11-temperature point × 5-time point). Each treatment was in duplicate (55 × 2 = 110 samples). Therefore, ten pieces of meat samples were placed at a time in a water bath in each temperature point. Then two samples (the duplicate) were removed at each time point and were cooled in running cold tap water for 10 min to minimize further cooking. Heat treated meat samples were removed from the vacuum packed plastic bags and dried using paper tissue. The final weight was recorded to calculate cooking loss. Finally, each cooked sample was cut into slices (5 mm-thickness) across the fiber direction and the slices were ready for Raman spectroscopy measurement.

Cooking loss was calculated by weighing the sample before and after heat treatment, as mentioned above, and expressed as a percentage of the original weight.

2.3. Differential scanning calorimetric (DSC) analysis

A raw pork meat sample (32.4 mg) was placed in a sample cell of a differential scanning calorimeter (DSC 1) (STAR® System, Mettler Toledo, Schwerzenbach, Switzerland) to follow the major endothermic transitions in the pork muscle. An empty sealed aluminum crucible was placed in the reference cell. The sample was scanned from 30 °C to 90 °C with a heating rate of 2 °C/min.

2.4. Raman spectroscopic measurement

Raman measurements were made on the new cut surface of the meat slices using the RamanRx1 instrument (Kaiser Optical Systems, Inc., Michigan, 132 USA). The slices were placed on a metal plate under a microscope with a 10× objective to collect the Raman scattering from the samples. The instrument was equipped with a 785 nm laser (Invictus, Kaiser Optical Systems Inc., Michigan, USA), a single holographic grating and a thermoelectric cooled charge-coupled device detector, operated at −40 °C. The laser was focused on each sample parallel to the muscle fiber direction, and the laser power on the sample was 120 mW. The spectra were acquired using an average spectrum of 2 scans each with an exposure time of 10 s and were stored as Raman shifts in the range of 1800–200 cm⁻¹. Four spectra were collected by making grids with a distance of 4 mm on the surface of each slice and the average of the 4 spectra was used for data analysis (110 samples × the average of four spectra per sample = 110 average spectra). Occasional Raman spectra of high fat content were removed during the measurement in order to focus on the structural changes of proteins.

2.5. Chemometrics

Raman spectra, cooking loss data, cooking temperature and cooking time were imported into Matlab software (MATLAB 2014a) (Mathworks, Massachusetts, USA) for data processing. The MatLab and Partial Least Squares (PLS) Toolbox (Version 7.5 Eigenvector Research, Inc.; Wenatchee, WA, USA) were used to perform principal component analysis (PCA) (Wold, Esbensen, & Geladi, 1987) and partial least squares (PLS) regression (Wold, Martens, & Wold, 1983) on the dataset. The Raman spectra were preprocessed using extended multiplicative signal correction (EMSC) (Martens, Nielsen, & Engelsen, 2003) and then mean centered.
3. Results

3.1. Differential scanning calorimetry

Fig. 1 shows a representative DSC thermogram of raw whole pork meat scanned from 30 °C to 90 °C with a heating rate of 2 °C/min. Three endothermic transitions namely at 53.4 °C, 61.6 °C and 76.1 °C were observed (Fig. 1). These three endothermic transition regions are in agreement with the literature (Xiong et al., 1987). These transition regions have been well known and are ascribed to myosin, sarcoplasmic proteins and connective tissues and actin, respectively (Tornberg, 2005; Xiong et al., 1987).

3.2. Raw Raman spectra

In this study, a relatively small sample dimension was used in order to achieve quick temperature equilibrium and obtain the information about the denaturation of meat proteins. The raw Raman spectra of samples cooked at different cooking temperatures (50–70 °C) for 4 h are presented in Fig. 2. The spectra are colored according to the cooking temperature in order to uncover any systematic difference in the raw spectra due to the cooking temperature. The spectra from samples cooked at 50 and 52 °C have high background fluorescence which even grows higher at 54, 56 and 58 °C then decreased as the cooking temperature was increased (Fig. 2). The same trend was observed for all the other cooking times.

3.3. Effect of cooking temperature and time-preprocessed Raman spectra of pork muscle

Preprocessed Raman spectra from the samples cooked at different cooking temperatures (50–70 °C) for 4 h are presented in Fig. 3A. Table 1 shows the assignments of Raman bands, indicated with numbers in the figure (Fig. 3A). The spectra show a gradual decrease in intensity in the regions of amide-I (1655–1650 cm\(^{-1}\)), CH-bending (1340 cm\(^{-1}\)) and C–C stretch (940–934 cm\(^{-1}\)) as the cooking temperature increases from 50 °C to 70 °C (Fig. 3B–D). On the contrary, a gradual increase in intensity at 1668–1663 cm\(^{-1}\) (amide-I = > \(\beta\)-sheet and random coil) and 1243–1237 cm\(^{-1}\) (amide-III = > random coil and \(\beta\)-sheet) are observed as the cooking temperature increases (Fig. 3B–C). Fig. 3B also shows that samples cooked below 60 °C had wider Raman peaks with lower intensity in the amide-I region whereas samples cooked especially at the maximum temperatures (68 and 70 °C) had a narrow peak in the amide-I region.

Furthermore, there was a decrease in intensity in the S–S stretching band as the cooking temperature increased from 50 to 70 °C (Fig. 3E).

3.4. Changes in local microenvironment

Both cooking temperature and cooking time proved to have a significant effect on the intensity ratio of the Tyr doublet (1656 cm\(^{-1}\)/1627 cm\(^{-1}\)) (Fig. 4 left and right, respectively). The intensity ratio was significantly higher in samples cooked at 54 °C than at all the other cooking temperatures (Fig. 4 left). The intensity ratio increased significantly as the cooking time increased (Fig. 4 right). The higher standard deviation in Fig. 4 (right) was due to the high effect of cooking temperature in each cooking time. There was no significant interaction effect of cooking temperature and cooking time on the intensity ratio of the Tyr doublet (Fig. not shown). The intensity ratio of Trp was calculated by dividing its intensity to the intensity of the CH\(_2\) bending band at 1450 cm\(^{-1}\) as the latter band has been expected not to change during conformational changes (Herrero et al., 2004). There was a significant effect of cooking temperature on the intensity ratio of Trp (1758 cm\(^{-1}\)/
Fig. 2. Raw Raman spectra (1800–400 cm\(^{-1}\)) from longissimus thoracis of pork cooked at different cooking temperatures (50–70 °C) for 4 h. Colored according to cooking temperature (n = 11). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Raman spectra preprocessed using extended multiplicative signal correction (EMSC) from longissimus thoracis of pork cooked at different cooking temperatures (50 °C–70 °C) for 4 h. Colored according to cooking temperature. Selected regions: 1800–400 cm\(^{-1}\) (A), 1680–1630 cm\(^{-1}\) (B), 1350–1220 cm\(^{-1}\) (C), 960–920 cm\(^{-1}\) (D) and 580–500 cm\(^{-1}\) (E). See Table 1 for the assignment of the labeled bands (n = 11). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
3.5. Discrimination of Raman spectra from meat samples — PCA calculation

3.5.1. Effect of cooking temperature

Fig. 6A shows the PCA-score plot for all the Raman spectra colored according to the cooking temperature. Two outliers were removed based on their high values in Hotelling’s $T^2$. The first principal component (PC-1) was able to show the general stepwise structural changes in the meat samples cooked at 50 to 70 °C (Fig. 6A). The loadings plot for PC-1 (Fig. 6B) reveals that the samples cooked above 60 °C were positively correlated to the high intensity of bands at the amide-I (1668 cm$^{-1} = > \beta$-sheet) and amide-III (1238 cm$^{-1} = > \beta$-sheet) regions. In contrast, a negative correlation was found for the bands from CH bending (1340 cm$^{-1}$), C – C stretching (α-helix), Tyr (856 and 827 cm$^{-1}$), Trp (around 756 cm$^{-1}$) and $S – S$ stretching (530 cm$^{-1}$). PC-2 scores discriminate the samples cooked at 50–52 °C from all the other samples in general and from samples cooked at 54–58 °C in particular (Fig. 6A). The loadings plot for PC-2 shows that there was a negative contribution from bands at 1642 cm$^{-1}$, 1340 cm$^{-1}$, 934 cm$^{-1}$ and 521 cm$^{-1}$, and a positive contribution from bands at 1668, 1236, 1010 cm$^{-1}$ (Fig. 6C).

3.5.2. Effect of cooking time

PCAs were calculated separately for each cooking temperature in order to investigate the effect of cooking time (2–10 h) at each cooking temperature. In general, these analyses showed no clear discrimination between the different cooking times (Fig. not shown). However, samples cooked for the different cooking times either at 62 or 64 °C showed a tendency to be discriminated in their respective PCA-score plot. The PC-1 vs PC-2 score plot (Fig. 7 left) shows that there is a tendency for a discrimination of samples cooked below and above 6 h at the cooking temperature of 62 °C. The corresponding loadings plot for PC-1 shows that samples cooked for more than 6 h at 62 °C were negatively correlated to a high intensity of bands at 1645 and 1332 and a region assigned to $S – S$ stretching (537 and 514 cm$^{-1}$) (Fig. 7 right). Although the peak was not well defined, there was also a contribution of the $C – C$ stretch (934 cm$^{-1} = > \alpha$-helix) (Fig. 7 right).

3.6. Prediction of cooking loss, cooking temperature and time

Both cooking temperature and time had a significant effect on cooking loss which is shown in Fig. 8. There was no significant interaction effect of cooking temperature and cooking time on cooking loss (Fig. not shown). Cooking loss increased significantly as cooking temperature and/or time increase (Fig. 8 left and right, respectively). PLS regression revealed that cooking loss can be predicted from the Raman spectra using 2 latent variables with a very good fit: the squared correlation coefficient ($R^2$) of 0.82 (Table 2). The root mean square error of cross validation (RMSECV) was 2.82% (Table 2). By inspection of the regression coefficients, a positive contribution mainly from $\beta$-sheet (1668 and 1238 cm$^{-1}$) and a negative contribution from $\alpha$-helix (1643 and 936 cm$^{-1}$) were revealed (Fig. not shown). Also, a negative contribution from the Tyr (around 856 and 827 cm$^{-1}$), Trp (around 756 cm$^{-1}$) and $S – S$ stretch (530 cm$^{-1}$) was revealed (Fig. not shown).

Prediction of cooking temperature using the Raman spectra showed very high correlation ($R^2 = 0.96$) with a prediction error (RMSECV) of 1.26 °C (Table 2). Four latent variables were required to predict the cooking temperature. In contrast, the PLS prediction of cooking time resulted in a poorer model ($R^2 = 0.78$) which required six latent variables (Table 2). Similar bands (around 1668, 1644, 1340, 1237, 937, 856, 827, 756 and 530 cm$^{-1}$) in LV-1 which contributed to the prediction of cooking loss were also the main contributors for the prediction of cooking temperature and time (Fig. not shown). Apart from this, there was a clear negative contribution from: 939, 855, 827, 756, and 536 cm$^{-1}$ in LV-2 for the prediction of cooking time which was not the case in the models for the prediction of cooking loss and cooking temperature.

4. Discussion

In this study, Raman spectroscopy was used to elucidate conformational changes in meat proteins during cooking. The higher background intensity in samples cooked at 54, 56 and 58 °C might be due to the

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Table 1

<table>
<thead>
<tr>
<th>Band nr.</th>
<th>Band position (cm$^{-1}$)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1685–1645</td>
<td>Amide-I ($\beta$-sheet, random coil and $\alpha$-helix)</td>
</tr>
<tr>
<td>2</td>
<td>1606</td>
<td>Tyrosine, phenylalanine, tryptophan</td>
</tr>
<tr>
<td>3</td>
<td>1554</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>4</td>
<td>1450</td>
<td>CH$_2$, CH, CH – all bending</td>
</tr>
<tr>
<td>5</td>
<td>1341</td>
<td>CH – bending</td>
</tr>
<tr>
<td>6</td>
<td>1309–1229</td>
<td>Amide-III ($\alpha$-helix, random coil and $\beta$-sheet)</td>
</tr>
<tr>
<td>7</td>
<td>1003</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>8</td>
<td>940</td>
<td>C – C stretching ($\alpha$-helix)</td>
</tr>
<tr>
<td>9</td>
<td>850</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>10</td>
<td>830</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>11</td>
<td>760</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>12</td>
<td>560–510</td>
<td>$S – S$</td>
</tr>
</tbody>
</table>

*Adopted from: Chen & Lord, 1974; Herrero, 2008; Wei et al., 2008.*

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Fig. 4. Mean relative intensity ratio of tyrosine ($I_{856\ cm^{-1}}/I_{514\ cm^{-1}}$) of longissimus thoracis of pork cooked at different cooking temperatures (left) (n = 110) and different cooking times (right) (n = 110). Different letters indicate significant differences (P < 0.05). Bars represent standard deviation of means.
effect of the dynamic process of denaturation of myosin in this range of temperature (Fig. 2). The unfolding of myosin molecules in turn leads to exposure of aromatic hydrophobic residues primarily Trp, phenylalanine (Phe) and Tyr (Boyer, Joandel, Ouali, & Culioli, 1996), which are known to cause fluorescence (Chan, Gill, & Paulson, 1992). This is confirmed in the present study where the intensity ratio of Tyr and Trp was significantly higher at 54 and 56 °C, respectively (Figs. 4 and 5) (see below for clarification). The reduced background at the higher temperatures (above 60 °C) (Fig. 2) might be due to the involvement of the hydrophobic residues in the formation of protein aggregates. In

![Fig. 5. Mean relative intensity ratio of tryptophan (I758cm⁻¹/\text{I1450 cm}^{-1}) of longissimus thoracis of pork cooked at different cooking temperatures (left) (n = 110) and different cooking times (right) (n = 110). Different letters indicate significant differences (\(P < 0.05\)). Bars represent standard deviation of means.](image)

![Fig. 6. Principal component analysis (PCA) of Raman spectra (1800–400 cm⁻¹) from longissimus thoracis of pork cooked at different cooking temperatures (50 °C–70 °C) for different cooking times (2–10 h). PC-score plot (PC-1 vs PC-2) colored according to cooking temperature (A) and PC-loadings plot for PC-1 (B) and PC-2 (C). PC-1 and PC-2 explained 87.25% of the variation in the Raman spectra (n = 108). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image)
a study on myosin solutions from fish, Chan et al. (1992) found that the fluorescence decreased as temperature increased during heat treatment from 22 to 60 °C. Furthermore, this study showed that the remaining background fluorescence (Fig. 2) was best compensated for by employing the EMSC preprocessing (Fig. 3A).

The preprocessed Raman spectra (Fig. 3A–E) reveal changes in frequency and intensity in the Raman bands which are useful for understanding the denaturation process of meat proteins. The changes are primarily indicators for changes in the secondary structure of the polypeptide backbone, disulfide bonds and in turn the microenvironment of the amino acid side chains. The Raman bands were assigned based on the literature (Chen & Lord, 1974; Herrero, 2008; Wei, Zhang, Halas, & Hartgerink, 2008). Model compounds/polymers have been used for the assignment of Raman bands (Chen & Lord, 1974; Wei et al., 2008) and it has been observed that it is difficult to find the exact position of the conformations especially in the amide-III regions (Chen & Lord, 1974). This could be due to different factors such as the strength of the hydrogen bond. Chen and Lord (1974) made assignments of Raman bands in the amide-I region as follows: α-helix (1655–1650 cm\(^{-1}\)), random-coil (around 1665 cm\(^{-1}\)) and β-pleated sheet (around 1666 cm\(^{-1}\)) and amide-III region as: α-helix (1300–1265 cm\(^{-1}\)), random coil (1253–1243 cm\(^{-1}\)) and β-sheet (1235–1229 cm\(^{-1}\)) using known polymers.

The same regions showed changes in position and/or intensity in the present study (Fig. 3B–C). The wider Raman peaks with a lower intensity in the amide-I region for samples cooked below 60 °C compared to a narrow peak for samples cooked at the maximum temperatures (68 and 70 °C) can also indicate the complexity of the system. The changes in position and/or intensity of the Raman band in this study are in good agreement with other studies which found an increase in β-pleated sheet and a decrease in α-helix in intact muscle (Beattie, Bell, Borggaard, & Moss, 2008), meat batters (Herrero, Carmona, Cofrades, et al., 2008; Herrero, Carmona, Lopez-Lopez, & Jimenez-Colmenero, 2008; Shao et al., 2011) and extracted myofibrillar proteins (Xu et al., 2011) during heat treatment. As pointed out above, the primary cause for the changes in intensity and frequency of bands (below 60 °C) might be the denaturation of myosin where changes in its structure lead to shifting position of bands in the amide-I (1650 cm\(^{-1}\)) and amide-III regions (Carew, Stanley, Seidel, & Gergely, 1983). Denaturation of connective tissues and sarcoplasmic proteins (Fig. 1) may also have contributed to the changes in frequency and intensity of the Raman bands as they denature mainly above 60 °C (Voutila, Mullen, Ruusunen, Troy, & Puolanne, 2007; Xiong et al., 1987). In relation to meat quality, a higher amount of β-sheet has been positively correlated to increased sensory toughness in beef (Beattie et al., 2004), and to

Fig. 7. Principal component analysis (PCA) of Raman spectra (1800–400 cm\(^{-1}\)) from longissimus thoracis of pork cooked at 62 °C for different cooking times (2–10 h). PC-score plot (PC-1 vs -2) colored according to cooking time (left) and PC-loadings plot for PC-1 (right) (n = 10).

Fig. 8. Mean cooking loss (%) of longissimus thoracis of pork cooked at different cooking temperatures (left) (n = 110) and different cooking times (right) (n = 110). Different letters indicate significant differences (P < 0.05). Bars represent standard deviation of means.
higher penetration force values in cooked meat batters (Herrero, Carmona, Lopez-Lopez, et al., 2008).

Disulfide bonds are known to stabilize the tertiary structure of proteins. Raman bands at the S – S stretching vibrations of disulfide bonds region: 508, 528 and 540 cm⁻¹ are assigned to gauche–gauche–gauche, gauche–gauche–trans and trans–gauche–trans, respectively (Sugeta, Go, & Miyazawa, 1973; Van Wart & Scheraga, 1986). Xu et al. (2011) found that the intensity of the S – S stretching around 545 cm⁻¹ was decreased as heating temperature was increased from 30 to 70 °C during gel formation of extracted pork myofibrillar proteins. They ascribed this to the conversion of the trans–gauche–trans conformations to the other conformations as the temperature increased. Therefore, the decrease in intensity at 530 cm⁻¹ (gauche–gauche–trans) and broadening of the band could indicate the loss of the native form of the disulfide bonds in the present study.

The intensity ratio for Tyr doublet (I₆₅₆ cm⁻¹/I₆₂₇ cm⁻¹) and for Trp (I₄₇₈ cm⁻¹/I₄₅₀ cm⁻¹) was calculated in order to investigate the change in the local microenvironment during the thermal treatment. In this study, the intensity ratio for Tyr was above 1 (Fig. 4) indicating that the Tyr residues were involved in hydrogen bonding by acting as both hydrogen acceptor and donor in a polar environment (Wei et al., 2008; Xu et al., 2011). It was interesting to see the significantly higher intensity ratio of Tyr at 54 °C which was the temperature where an endothermic peak assigned to myosin was observed (Fig. 1). The decrease in the intensity ratio of Tyr and Trp as the temperature was increased might be due to the involvement of these residues in hydrophobic interactions after they have been exposed to the aqueous environment in the higher temperature. Our results are in agreement with previous work (Xu et al., 2011) who found similar trends during heat-induced gel formation. The increase in intensity ratio of the Tyr doublet as a function of time might be due to a positive effect of cooking time on the polar microenvironment.

PCA-score plot (Fig. 6A) revealed the systematic conformational changes in the meat proteins discussed above. In general, inspecting the score and loadings plots (Fig. 6), PC-1 reflects the increase in random coil and/or β-sheet structure and decrease in disulfide bond as the temperature increases from 50 to 70 °C whereas PC-2 reveals that there was a decrease in α-helix structure and a start for the increase in random coil and β-sheet structures below 60 °C. PC-2 also explains a loss in the gauche–gauche–trans conformation as the temperature increased. With regard to the effect of cooking time (Fig. 7), it could be suggested that such clustering of the Raman spectra at the specific cooking temperatures (62 and 64 °C) might be due to structural changes in connective tissues during the extended cooking time which favors collagenase (Tornborg, 2005). Similarly, an endothermic peak assigned to sarcoplasmic proteins and connective tissues was observed in the same temperature range in the DSC thermogram (Fig. 1). The higher intensity ratio of both Tyr and Trp at 64 °C compared to the other temperatures higher than 60 °C (Figs. 4 and 5) can support our suggestion. In general, the absence of a clear effect of cooking time in most of the cooking temperatures seems to be in accordance with other objective measurement results (Christensen, Erhtuberg, et al., 2011) where increased cooking time from 3 to 8 h at cooking temperatures of 48, 53, 58 and 63 °C did not affect the shear force value of the longissimus dorsi of pigs. However, it is worth mentioning that prolonged cooking time (above 10 h) could affect shear force value of longissimus dorsi from pork (Christensen, Erhtuberg, et al., 2011). The novelty of the present study is, therefore, that Raman spectroscopy can provide such basic information about the stepwise changes in the secondary structure and in turn the hydrophobic environment in intact meat rapidly without following tedious procedures (homogenization, incubation, extraction etc.) and without using chemicals. The structural changes in the connective tissues were not pronounced as compared to the changes in myosin. This could be due to the small amount of connective tissue in the muscle (Christensen, Erhtuberg, et al., 2011) and/or other reasons. Therefore, further investigation is needed to study the structural changes in connective tissues during heat treatment using muscles with a high amount of connective tissues at higher temperatures.

The increase in cooking loss as a function of cooking temperature and/or cooking time is in good agreement with the literature (Christensen, Bertram, et al., 2011; Christensen, Erhtuberg, et al., 2011; Martens et al., 1982; Palka & Daun, 1999). This parameter has a negative correlation with the juiciness of a cooked product (Martens et al., 1982). Interestingly, previous work by Palka and Daun (1999) reported the highest increase in cooking loss in the range of 50 to 60 °C which in this study was particularly pronounced when the temperature increased from 56 to 58 °C (Fig. 8). The very good fit (R² = 0.82) (Table 2) of the model to predict the cooking loss shows the robustness of the model and is in good agreement with previous works (Beattie et al., 2008; Schmidt et al., 2013). The positive contribution of β-sheet can indicate that the structural changes in the meat proteins both in the myofibrils and connective tissue were the causes for the loss of water holding capacity of the myofibrillar proteins (Palka, 1999). It was interesting to see the contribution of the same regions (1668, 1642, 1340, 1235, 1009, 937, 758 and 520 cm⁻¹) of the Raman spectra for the prediction of cooking loss, temperature and time though there were differences in higher latent variables and the models used different numbers of latent variables. This can indicate the general interrelationship among these parameters with denaturation of meat proteins.

5. Conclusion

This study demonstrated that Raman spectroscopy can provide valuable information about stepwise conformational changes in the secondary structure of meat proteins, disulfide bond and in turn the hydrophobic environment at the molecular level in intact muscle. The effect of cooking temperature on the structure of meat proteins was pronounced compared to the cooking time. Raman spectra from samples cooked below and above 60 °C were discriminated. Bands at amide-I, amide-III and disulfide bond regions were found to be the most important spectral regions for the discrimination. Significant changes in the intensity ratio of bands from Tyr and Trp residues were found indicating changes in the hydrophobicity of the microenvironment during the thermal treatment. Cooking time did not show a clear effect in most of the cooking temperatures. It was possible to predict cooking temperature and cooking loss with a very good correlation and also cooking time using Raman spectra and PLS regression. The potential of Raman spectroscopy to predict cooking temperature could be of potential interest for the meat industry for quality control as it is possible to predict the core temperature of a given cooked meat product after it has been cooked and chilled.

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