USE OF RAMAN SPECTROSCOPY TO STUDY EFFECT OF COOKING TEMPERATURE AND TIME ON MEAT PROTEINS

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Abstract – The aim of this study was to investigate potential of Raman spectroscopy to study effect of cooking temperature and time on meat proteins. Pieces of pork *Longissimus dorsi* muscle samples were heat treated in a water bath at different cooking temperature for different cooking time. Raman spectra were collected by exciting the meat samples using a Raman microscopy with an excitation wave length of 785 nm for 10 sec and 2 accumulations. It was found that meat samples cooked at different cooking temperature were discriminated when principal component analysis (PCA) was carried out on pre-processed Raman spectra. The Raman spectra provided information about the degree of protein denaturation during cooking at different cooking temperatures and it was clear from the PCA plot that cooking temperature had more effect on protein denaturation than cooking time. This study demonstrated that Raman spectroscopy has a potential to provide information about the changes in meat proteins during heat treatment.

Key words - Chemometrics, Raman spectra, Thermal treatment

I. INTRODUCTION

Proteins are, beside water, the major component of meat: water (~ 75 %), protein (~ 20%), fat (~2%) and minor components such as carbohydrates, minerals etc. (3%). Proteins are important to the structure of meat and processed meat products [1]. The structural changes of proteins therefore, have an effect on the quality of a product [2].

Heat treatment of meat affects structure of the meat proteins [3]. There are mainly two determinant factors which need an attention when dealing with heat treatment namely: cooking temperature and time [4], [5]. Effect of these two factors on structure of meat proteins [6] and other quality parameters including sensory quality [4], [5] has been studied intensively. For example Christensen *et al*. [6] studied the denaturation of meat proteins under Low-Temperature Long-Time (LTLT) using Differential Scanning Calorimetry (DSC) and Low-field Nuclear Magnetic Resonance (LF-NMR). Although these methods can give valuable information about the denaturation of the meat proteins[6], [8], they have their limitations. For example, they are not well-suited for routine activity in the meat industry. Therefore, there is a need to examine other methods which could be used in the industry for non destructive routine on-line analysis.

Raman spectroscopy can be used to monitor quality of meat in the meat processing industry and hand-held instruments are already in use for research [9]. Although LF-NMR could provide information about the states of water related to peptides and proteins, it might not be useful to study the changes in protein structure. In contrast to Infrared (IR) spectroscopy, Raman spectroscopy has the advantage that it is relatively insensitive to water which is useful in relation to meat as meat contains approximately 75 % water.

Thermal treatment is one of the factors which can affect the quality of meat. Good correlations between Raman data from cooked meat and shear force and also cooking loss percentage have been reported [8] using a fixed cooking temperature (E.g 70 ^{o}C). However, it is important to examine the dynamic process during thermal treatment using Raman spectroscopy as structural changes in meat proteins is not one step process [1]. The objective of this study was to use Raman spectroscopy to study protein structural changes due to effect of cooking temperature and time.

II. MATERIALS AND METHODS

Three female pigs with a slaughter weight of 74.3 to 77 kg were slaughtered in a slaughter house (UCR, Roskilde). *Longissimus dorsi* (LD) muscle was sampled at the position between the 9th and 13th ribs at 24 h post- mortem. The muscle was cut into chops, vacuum packed and stored at -20 ^{o}C until use. Frozen chops were thawed at 4 ^{o}C for 15 hr and cut into small pieces of samples. Weight of each sample was recorded. The samples were then re-vacuum packed separately and heat treated in a pre-heated water bath (ICC Denmark) at different cooking temperatures (50,54,58 ^{o}C) for different cooking time (2, 4, 6, 8 and 10 h). Each treatment had duplicates. The final weight of each piece was recorded after cooking to calculate cooking loss in percentage.

Raman measurements were made using RamanRxn1 instrument (Kaiser Optical Systems Inc, Michigan,132 USA) on a new cut surface of the cooked meat 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 CCD detector, operated at -40°C, were used. Mk II filtered probe attached to a microscope with a 10x objective was used to collect the Raman scattering from the samples. The spectra were acquired using an exposure time of 10 s with number of accumulations of 2 and were stored as Raman shifts in the range 1800 - 200 cm^{-1}. Four spectra were collected from each piece of meat.

All Raman specra were preprocessed using 2nd derivative Savitzky-Golay and mean centered before performing Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression with MatLab software (MATLAB 2011b) (Mathworks, Massachusetts, USA) and PLS Toolbox (Version 7.0.2 Eigenvector Research, Inc.; Wenatchee, WA, USA).

III. RESULTS AND DISCUSSION

Raman spectra preprocessed using 2nd derivative Savitzky-Golay were coloured according to cooking temperature (Figure not shown). The changes in meat proteins were revealed in the amide-I (1672-1650 cm^{-1}) and- III (1275-1225 cm^{-1}) regions and in the region of the spectra assigned for Tyrosine doublet (at 855 and 830 cm^{-1}).

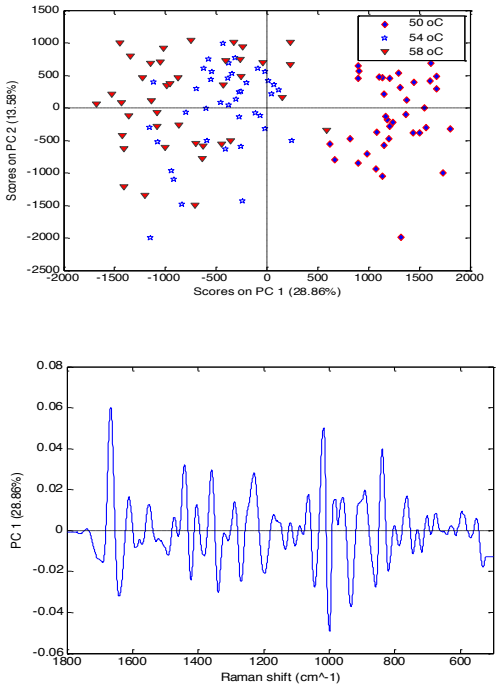

Figure 1. Principal component analysis (PCA) of Raman spectra (1800-500 cm^{-1}) from *Longissimus dorsi* muscle of pork cooked at different cooking temperature (50, 54 and 58 ^{o}C) for different cooking time (2-10 h). PC-score plot (PC-1 vs -2) coloured according to cooking temperature (top) and PC-loadings plot for PC-1 (bottom).

PCA was performed to test if Raman spectroscopy could be used to discriminate the samples according to the cooking temperature and time (Fig. 1). It was found that there was a systematic variation among the samples cooked at the different cooking temperatures. Principal component number 1 (PC-1) is able to discriminate the samples cooked at different cooking temperatures when the full Raman spectrum (1800-500 cm^{-1}) was used for the analysis. The samples cooked at the low temperature (50 ^{o}C) are clearly separated from the other samples. This could be due to the reason that denaturation of myosin (rods and light chain) took place at the temperature of 54 ^{o}C [8].

The loadings plot (Fig. 1(bottom)) revealed that the change in the amide I region was the main contributor for such discrimination in the score

plot. This could be due to the reason that the conformation in secondary structure of the proteins was increased as the cooking temperature was increased. This is in agreement with others work who found an increase in β-sheet and a decrease in α-helix in intact muscle [10] and meat batters [11] as cooking temperature was increased. Such extra information is an advantage of using Raman spectroscopy as compared to using DSC to study denaturation of meat proteins.

There was no clear discrimination of samples due to the effect of cooking time in each cooking temperature (Figure not shown). In contrast, Mortensen *et al*. [5] found that cooking time had an effect on some sensory quality parameters in beef such as boiled veal aroma, boiled veal flavor, brothy aroma. The potential explanation for this could be that the molecules responsible for such parameters might be in small concentration in the samples where Raman spectroscopy is not sensitive for them [12].

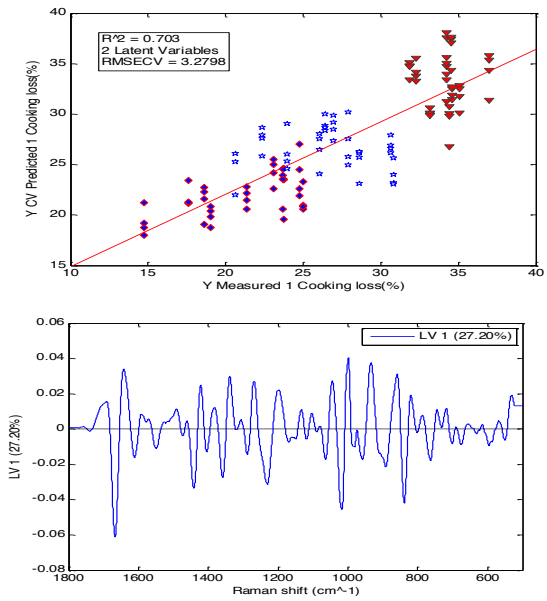

Figure 2. Partial Least Squares (PLS) regression for cooking loss predicted from Raman spectra (1800-500 cm^{-1}) (Top) and regression coefficient for a PLS model (latent variable-1) used for prediction (bottom)

In the present study, the minimum and maximum cooking loss percentages were 15% at 50 ^{o}C cooked for 2h and 37% at 58 ^{o}C cooked for 10h, respectively (Fig. 2 (Top)). Figure 2 showed that it was possible to predict cooking loss from the Raman spectra using 2 latent variables with coefficients of determination (R^2) of 0.70. The Root Mean Square Error of Cross Validation (RMSECV) was 3.27 % which showed a good performance of the developed regression model for the prediction of the cooking loss. The R^2 value in this study was almost similar to others work where the R^2 value was about 0.78 in pork muscle [9]. These results indicate that Raman has the potential to predict cooking loss. The latent variables were plotted and it was found that the most important region for the prediction was the amide I region (1667 cm^{-1}) which is assigned for β-sheet structure (Fig. 2 (bottom)). As discussed above, the change in amide-I region can be considered as an indicator for the denaturation of meat proteins.

IV. CONCLUSION

This study showed that Raman spectroscopy can reveal an ongoing process in meat proteins during thermal treatment even at low cooking temperatures. It was also possible to use Raman spectra to predict cooking loss which is an indirect indicator for the denaturation of meat proteins. There was no clear discrimination among samples cooked for different cooking time at the same cooking temperature. Raman spectroscopy can, therefore, be used to prepare a cooking temperature profile in the heat treated meat producing industry.

ACKNOWLEDGEMENTS

This study is part of a PhD-project entitled as 'Raman Spectroscopy- Process Analytical Technology (PAT) for quality monitoring in the meat processing industry' funded by the Danish Meat Research Institute (DMRI) and University of Copenhagen. We thank Franciscus Winfried J van der Berg, Carl Emil Aae Eskildsen and Claus Borggaard for their discussion during the data analysis.

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