INFRARED SPECTROSCOPIC DIFFERENTIATION OF THE EFFECTS OF COOKING AND ACIDIC TREATMENT IN MEAT

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Abstract – Meat is exposed to heat and acid pH during technological processing and digestion, respectively. Both treatments affect the molecular structure of meat proteins. In order to characterize separately the effects of heating and acidic treatments, infrared (IR) spectra of treated meat were analyzed. IR spectra of muscle fibers were obtained from histological sections of raw and cooked meat which were incubated in 0.9% NaCl solution adjusted to pH 2, 3.5 and 5 using a transmission IR microscope. Despite the low thickness of the sections (6 µm), the Amide I and II bands' signal were saturated: There is the possibility of having a poor linearity in quantifying these band intensities. From this reason, the spectral region of the amide vibrational modes (1700-1500 cm^{-1}) was excluded from the data analysis. Another fingerprint region (1500-1000 cm^{-1}) was applied to the analysis. Results showed that the band corresponding to the protonation of Aspartic acid highlighted the effect of pH separately from the effect of cooking. However, bands which can well explain the cooking effect were not found in this spectral region.

Key Words – protein denaturation, muscle fiber, multivariate analysis, protein structure, infrared spectroscopy.

I. INTRODUCTION

Meat is exposed to heat and acid conditions during the processes of cooking and digestion respectively. The meat proteins undergo substantial structural changes following heat and acidic treatments and the changes affect the eating quality and digestibility of meat.

The effects of heat and acidic treatment on the structure and chemical state of proteins have been well studied. Thermal denaturation of beef muscle proteins was evidenced using infrared microspectroscopy [1]. The acid pH effect on the protein structure and the protonation of amino acid residues has also been investigated using IR and some other spectroscopic techniques [2].

The changes in meat IR spectra after cooking and acidic treatment are basically similar since cooking and acid treatment both denature protein. Separation of the effect of these two treatments is helpful to understand their consequences on eating quality and digestibility of meat which have been cooked and have undergone an acidic treatment. This study aimed to characterize the effects of cooking and of acidic treatment on the molecular structure of meat protein in order to better understand the role of protein structural changes on their digestion.

II. MATERIALS AND METHODS

Samples

Deltoid muscle (ca. 2.5 kg) from the both right and left sides of a beef carcass (Charolaise breed, 90 months old) were prepared and vacuum packed. Four days after slaughtering, one muscle was stored 10 hours under vacuum in a cold room (4 °C) while the controlateral muscle was vacuum cooked at 85°C for 10h. After cooking, the muscle was cooled down to 3°C. Both raw and cooked muscles were then stored at 4 °C until use.

On the same day of the cooking, raw and cooked deltoid muscles were cut into 1 cm × 1 cm × 2 cm pieces with the long axis parallel to the long axis of muscle fibers.

Every meat sample was put in a 60-mL test tube and incubated with 50 mL of 0.9% NaCl solution which was adjusted to pH 2, 3.5 or 5 with

aqueous HCl, and shaked for 2h. Three sample test tubes were used for each pH condition (triplicates). After 2 h incubation, meat samples were cryofixed in cooled isopentane (–160 °C) and stored at –80°C.

The cryofixed samples were cryosectioned transversally to the muscle fibers (6 µm thickness) and thaw-mounted on BaF_2 windows consistent with infrared microspectroscopy. The sections were vacuum packed and stored at –20 °C until measurements to avoid sample autoxidation.

IR Spectral Measurement

FT-IR acquisitions were performed with an IR microscope (Nicolet iN10, ThermoFisher). The spectra were recorded from single fibers in the region between 4000 and 600 cm^{-1} with a spectral resolution of 4 cm^{-1} and a spatial resolution of 20 µm × 20 µm. For each spectrum, 128 scans were accumulated and averaged.

From each sample section, about 40 spectra were acquired from different single fibers which were located at the edge of the meat samples and supposed to have the direct effect of the solution pH. A total of 734 spectra (about 40 fibers × 3 pH treatments × 3 replications × 2 cooking treatments) were obtained.

Data Analysis

To analyze the variation in the spectral data set and the correlation between the variation and the cooking and pH treatments, the principal component analysis (PCA) and the partial least-squares regression (PLSR2) were performed using The Unscrambler software version 9.8. In order to exclude the effects of fluctuation in baseline and sample section thickness, spectra were treated by extended multiplicative signal correction (EMSC) and second derivative function.

III. RESULTS AND DISCUSSION

Table 1 shows the final pH values of the acid solution after 2h incubation with the meat. The final pH increased after the incubation in all treatments probably because of the buffering capacity of skeletal muscle. Samples incubated in pH 3.5 solution showed the biggest increase both in raw and cooked treatments.

Typical as-observed spectra of the muscle fibers from raw and cooked beef samples are shown in Fig. 1. The intensity of absorbance in Amide I and II region (1700-1500 cm^{-1}) was considerably higher in cooked samples than raw samples and there was the possibility of having a poor linearity in quantifying these band intensities. From this reason, this spectral region was excluded from the data analysis despite the relevance of these bands to characterize the protein denaturation. The fingerprint region (1500-1000 cm^{-1}) was applied to the analysis in this study.

Fig. 2 presents the PCA score plot using the second derivative spectral data. The samples clustered according to the treatments and the PCA clearly separate the effect of pH 2. PC1 and PC2 explained 23% and 8% variance respectively and their loadings are shown in Fig. 3.

Table 1. Final pH values of the meat incubation saline after 2h (average ± s.d.).

Initial pH	Final pH	
	raw meat	cooked meat
pH 2	2.33 ± 0.04	2.18 ± 0.02
pH 3.5	4.96 ± 0.01	5.50 ± 0.06
pH 5	5.50 ± 0.03	5.85 ± 0.01

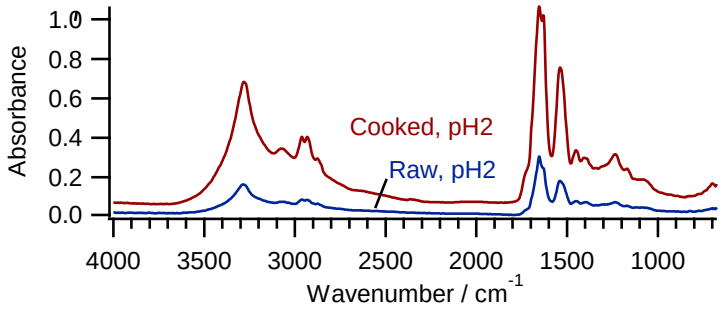

Fig. 1. Typical spectra of raw and cooked beef samples

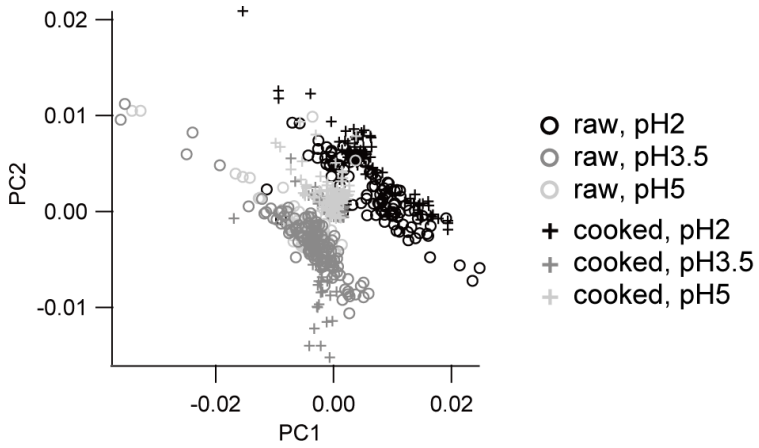

Fig. 2. Score plot of the PCA analysis of the second derivative spectral data of 1500-1000 cm^{-1} region.

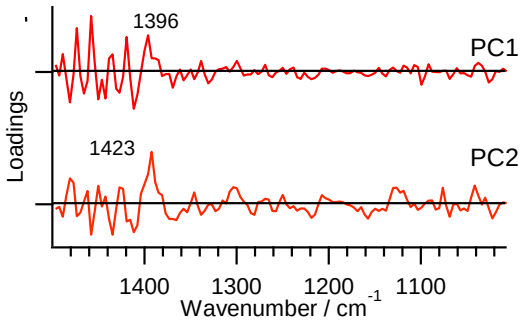

Fig. 3. Loading plots of the principal components obtained by the PCA analysis of second derivative spectra.

PLSR2 analysis was then conducted to elucidate the relationship between the spectral changes and pH and cooking. As the result, it was found that the first and second PLS component were able to separate the effects of cooking and pH 2. To visualize the effect of pH 2 and cooking treatments on the spectra, the correlation loading plot of the two PLS component is shown in Fig. 4.

The pH2 treatment was positively correlated with the bands at 1396 cm^{-1} (Fig. 4 upper). This band assigned to the symmetric stretching vibrations of the COO^- group provides the evidence of the Asp group protonation process in acid pH [2]. IR spectra could detect the all-or-non transition in protein chemical structure at the pK_a value in raw and cooked meat. Aspartic acid pK_a is 3.90. This can be a reason for incapability in detecting the difference in pH 3.5 and 5 treatments: Final pHs of both treatments were above 3.9 (Table 1).

Some bands which correlate with cooking treatment were identified (Fig. 4 lower). One of them is 1423 cm^{-1} which is attributed to aromatic residues of proteins and positively correlates with cooking. These bands, however, have only weak explanation ability (< 50%) of spectral data variance.

No band was found to explain the variables originate from pH 3.5 and 5 treatments (Fig. 4 lower). The spectral region other than 1500-1000 cm^{-1} should also be investigated to improve the separation of the effects of acid pH 3.5 and 5. The O–H and C–H stretching region (3500-2800 cm^{-1}) may have significant information.

IV. CONCLUSION

Some bands were identified that allow the cooking and acidic treatment (pH 2) characterizations in meat. However, the molecular changes caused by an incubation of meat at pH 3.5 to 5 were not evidenced by the 1500-1000 cm^{-1} finger print region. Other spectral regions will be investigated for the better separation of the effects of which meat expose during the process of consumption and digestion.

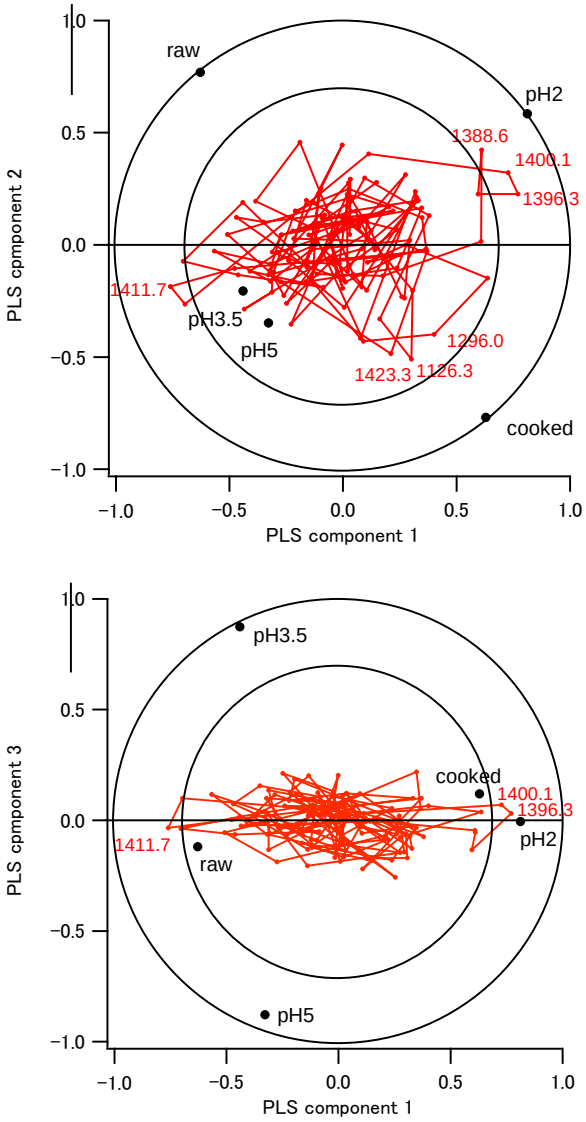

Fig. 4. Correlation loading plot of the PLS components. The inner and outer ellipses refer to 50% and 100 % explained variance.

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