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THE CHANGES OF PROTEIN STRUCTURE IN TILAPIA SURIMI DURING GELATION BY RAMAN SPECTROSCOPY

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ABSTRACT

Structural changes, textural properties in Tilapia surimi myofibrillar protein during gelation were studied by Raman spectroscopy. The change in the amide I (1600-1700 cm^{-1}) region indicated that the decrease in α-helices content accompanied by increase in ß-sheet and random coil after heating. The conformation of S-S bond was observed in the Raman spectrum near 500-600 cm^{-1} in the samples of 30-40 ^{o}C incubation temperature which produce textural profile with high gel strength. Intensity of the band near 758 cm^{-1} as well as a slight decrease in I853/I826 ratio when the heat increase 60-70 ^{o}C showed that the hydrophobic interaction was involved in the heat-induced gelation of surimi protein.

Keywords: tilapia surimi, protein structure, Raman spectroscopy.

1. INTRODUCTION

Surimi is fish mince that was washed by different washing solution and number of washing cycle, dewatered and mixed with cryoprotectant. Surimi based product is the jelly-like food made by heating surimi after grinding with 2-3% salt. Kamaboko, fish ball, fish cakes and fish sausage are place in this category. Quality of surimi is evaluated based on appearance, flavor, and texture. Texture is the most important factor to evaluate surimi-based products. The texture of product is formed by the gel-forming process of myofibrillar protein. When myofibrillar proteins have highly reactive surfaces once the protein is unfolded (denatured). During heating of salted surimi pastes, the proteins unfold, exposing reactive surfaces of neighboring protein molecules, which then interactive to form intermolecular bonds. When sufficient bonding occurs, a three-dimension network is formed, resulting in a gel.

Raman spectra can provide information on the micro-environment and chemistry of protein side chains as well as on the conformation of the protein polypeptide backbone [1]. The changes in Raman bands give information on modifications in secondary structure of protein (amide conformation region, C-C stretching vibration) and also in local environments (tryptophan residues, tyrosine doublet, aliphatic amino acid bands). Based on Raman studies of structural

changes of protein in Pacific whiting surimi, and during different gelling conditions, Bouraoui *et al.* (1997) [2] proposed that protein changes observed in the Raman spectra may be related to gel strength and fold score. The changes in intensity of Raman band near 530 cm^{-1} were attributed to either involvement of disulfide bond. Changes in secondary structure were more pronounced after cooking treatment. Other features, included changes in the tyrosine doublet ratio, frequency downshifting as well as intensity decease of the Raman band assigned to C-H stretching vibration of aliphatic residues.

The objective of this work was to study the structural change of myofibrillar protein of Tilapia surimi during gel forming process.

2. MATERIAL AND METHODS

2.1. Materials

Frozen Tilapia surimi was obtained from Viet Truong Company at Hai Phong. Samples were packed in a polystyrene box and immediately transported to the laboratory. Frozen surimi was cut into 1 kg blocks. Surimi was vacuum-packed, and kept at -18 ^{o}C until being used.

Tilapia surimi quality was evaluated and published by company as follow: Water: 76 %; protein content: 14.4 %; lipid content: 0.74 ; pH: 6.5, gel strength: 405 g.cm; whiteness: 84.25 %.

2.2. Methods

2.2.1. Preparation of gels

Frozen surimi was thawed at room temperature for 1 h and cut into small pieces and an appropriate amount was weighted and transferred to the cutting mixer machine. The surimi was then chopped for one min. Salt (2 %) and ice (final moisture content of surimi was adjusted to 80 %) was added and chopping step continued for 2 min more. The temperature was maintained bellow 10 ^{o}C at all times. The paste was carefully stuffed into PE case (30 cm length, 3 cm diameter), sealed and incubated in water bath for setting. Suwari gels were obtained by heat-setting at the temperature of 30, 40, 50, 60, and 70 ^{o}C for 30 min. Kamaboko gels were obtained by cooking suwari samples at 90 ^{o}C for 15 min in water bath. The gels were cooled immediately for 15 min to stop any further action of the heat in ice water. Gel strength test was carried out within a day of gel production.

2.2.2. Gel strength (GS) measurement

The refrigerated samples were allowed to reach room temperature (20-25 ^{o}C) prior to testing by texture analyzer Model TA.XTplus. Samples were cut to a length of 30 mm with a knife. Texture profile analysis was done using a spherical probe (Probe, N0 P/5S) at room temperature (20 ^{o}C), flowed by procedure using a texture analyzer. The machine was set up at 75 % deformation with 1 mm/sec crosshead speed, and 5 kg full scale. Each measurement was replicated 5-6 times.

2.2.3. FT-Raman spectroscopy

Raw frozen samples and surimi gel were stored at 4 °C until tested. Samples were thawed at 4°C for 10 min before examining by a visible laser Raman spectrometer (LabRAM HR 800, HORIBA Jobin Yvon, France). Samples were placed in a capillary tube and held horizontally in the spectrometer, thermostated at 4 °C. The incident laser beam was vertical, i.e., perpendicular to the capillary axis. Raman spectral data were collected on the Raman spectrometer with 612 nm excitation from an argon ion laser (He-Ne laser), cooled with the Coherent Laser Pure heat exchanger system. The conditions used were as follows: incident laser power of 100 mW, slit height of 4 mm, spectral resolution of 5.0 cm^{-1} at 19 000 cm^{-1}, sampling speed of 120 cm^{-1} min^{-1} with data collected every 1 cm^{-1}. Frequency calibration of the instrument was performed daily using the 1050 cm^{-1} (($1\ cm^{-1}$) band of a 1 M KNO 3 standard solution. To increase the signal-to-noise ratio, at least six scans of each sample were collected to obtain averaged spectral data. Triplicate samples of each treatment were scanned.

Spectra were smoothed; baseline corrected, and normalized against the phenylalanine band at 1007 cm^{-1} by GRAMS/32 Spectral Notebase version 4.14 level II (Galactic Industries Corp., Salem, NH). Assignments of peaks in the Raman spectra to specific vibrational modes of amino acid side chains or the polypeptide backbone were made according to published literature.

3. RESULTS AND DISCUSSION

3.1. Effect of setting conditions on texture of suwari and kamaboko tilapia surimi gels

Texture is the most important factor to evaluate surimi-based products. The texture of product is formed by the gel-forming process of myofibrillar protein. During setting process of salted surimi pastes, the proteins unfold, exposing reactive surfaces of neighboring protein molecules, which then interacts to form intermolecular bonds. When sufficient bonding occurs, a three-dimension network is formed, resulting in a gel. The effect of various incubation temperatures on the texture of suwari gels and kamaboko gels is presented in Fig. 1.

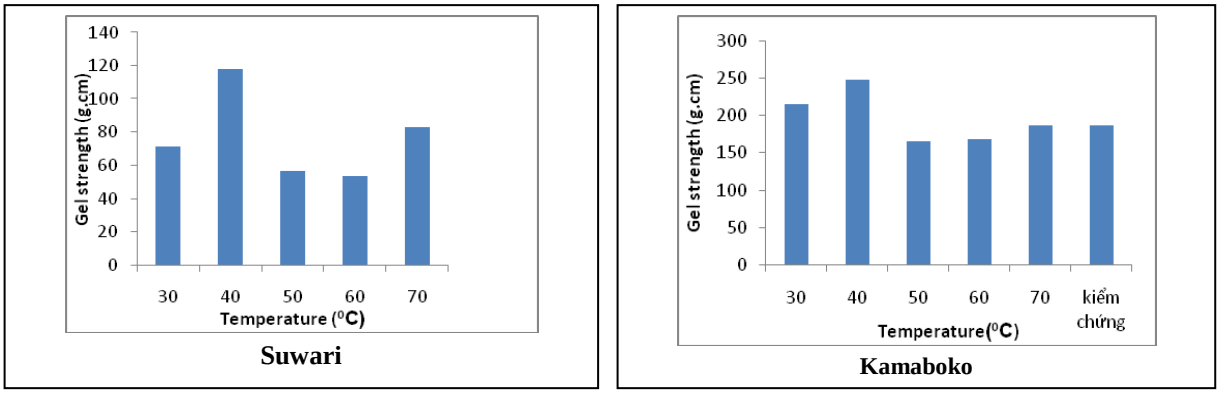

Figure 1: Effect of incubation temperature on suwari and kamaboko gel texture of tilapia surimi.

Setting surimi at the medium temperature (30-40 °C) resulted in increased of gel strength of both suwari and kamaboko gels. Without setting, suwari gels had no measurable force and deformation. In suwari and kamaboko gels, gel strength value peaked at 40 °C.

3.2. Changes of protein structure

The Raman spectra for suwari and kamaboko gels were observed in the 400-3500 cm^{-1} region. The frequency and intensity changes in the Raman bands were indicative of changes in the secondary structure and variations in local environments of Tilapia surimi proteins.

3.2.1. Changes of disulfide bonds

In the presence of multiple groups -SH and disulfide bonds (-S-S-) the gel is irreversible by heating, strong and durable. The bands generated by disulfide and SH bond, fall in the 500-650 cm^{-1} range. The Raman spectra show a weak band at 548-576 cm^{-1} region at 30 and 40 ^oC of incubation temperature (Fig. 2).

This Raman band almost disappeared in those of high temperature. Protein and peptide containing cystine residue usually show a band in the Raman spectrum near 500-600 cm^{-1} which has been assigned to S-S stretching vibration of disulfide bonds [3]. The conformation of S-S bond producing high gel strength.

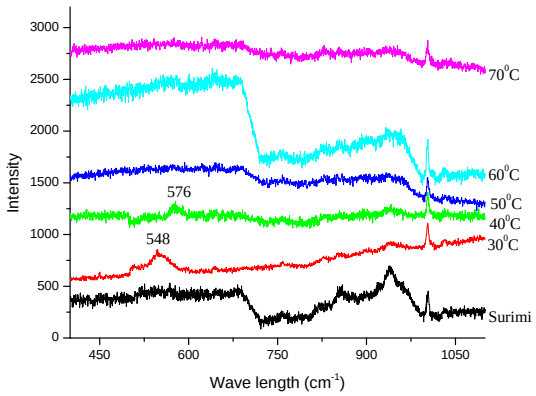

Figure 2: Raman spectra in the 400-1100 cm^{-1} region of Tilapia surimi at different incubation temperature.

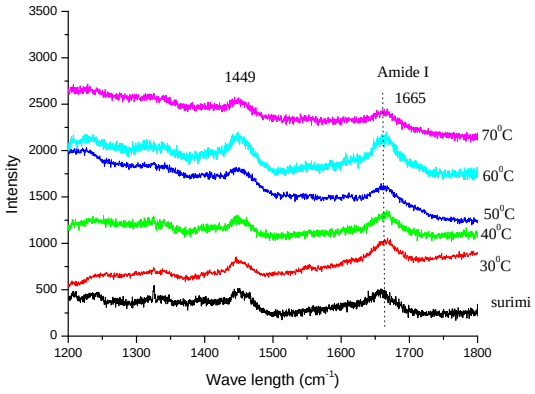

Figure 3: Raman spectra in the 1200-1800 cm^{-1} region of Tilapia surimi at different incubation temperature.

The most important Raman band to determine protein secondary structure is amide I band (1645-1685 cm^{-1}) and amide III (1200-1350 cm^{-1}) [3]. Fig. 3 shows the amide I band spectra in surimi and gel samples. In the Raman spectrum of Tilapia surimi heat-induced gelation in the 1600-1800 cm^{-1}, the most prominent band centered near 1655 cm^{-1}, has been assigned to the amide I vibrational mode [81], which involves mainly C=O streching and, to lesser degree, C-N streching, C_α-C-N bending, and N-H in plane bending of peptide groups. In general, the amide I band consists of overlapped band components falling in the 1658-1650, 1680-1665, and 1665-1660 cm^{-1} range, which are attributable to α-helices, ß-sheet, and random coil structure [4].

The amide I band at 1656 cm^{-1} in surimi corresponding to α-helice structure. The shift of the band was observed when heating. The bands in the range 1661-1665 cm^{-1} were observed in samples incubating at 30-50 ^oC, mainly involving an increase of random coil. We observed the band in the range 1668 cm^{-1} from samples when the temperature from 60-70 ^oC. This spectra change could be caused by ß-sheet formation.

3.2.3. Changes of local environments

Many Raman bands such as those at 760, 879, 1336, 1359, 1363, and 1557 cm^{-1} display information about the microenvironment of the tryptophan residues. Aromatic amino acid side chains show several characteristic Raman bands, some of which are useful to monitor the polarity of the microenvironment, or involvement in hydrogen bonding. If tryptophan residues from a buried, hydrophobic microenvironment become exposed to the polar aqueous solvent, there may be decrease in the intensity of 760 cm^{-1} band [3]. The intensity of the band near 760 cm^{-1} did not observed in surimi gel when heating at 30 and 40 ^{o}C. The band at 758 cm^{-1} was appeared when the heat increase in the range 50-70 ^{o}C, which indicated that the hydrophobicity was involved in the heat-induced gelation of tilapia surimi protein (Fig. 2).

The doublet bands located near 830 cm^{-1} and 850 cm^{-1} can be useful in monitoring the microenvironment around tyrosyl residues. In the case of tyrosine residues which are exposed to the aqueous or polar environment or which act as simultaneous acceptor and donor of moderate to weak hydrogen bonds, the intensity ratio of the doublet bands usually ranges from 0.90 to 1.45, but can be as high as 2.5. If I850/I830 value for tyrosine residues which are buried in a hydrophobic environment and which trend to act as hydrogen donors usually range 0.7-1.0 [1].

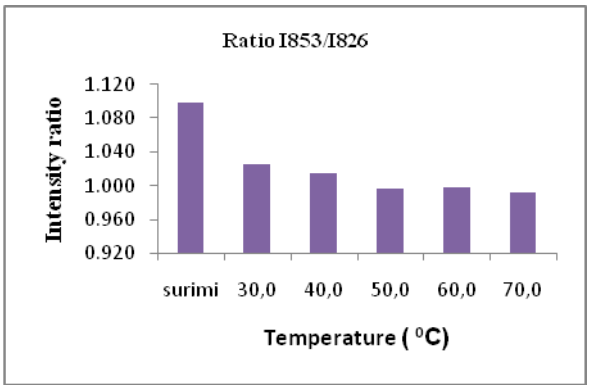

Figure 4. Tyrosine doublet ratio I850/830 in Tilapia surimi gel.

The results from Fig. 4 showed that the ratio I853/826 ranged from 0.992 to 1.099 during the Tilapia myofibrillar protein heat-induced gelation, which suggested that there is the conformation of weak hydrogen bonds. A slight decrease in the I853/I826 ratio was observed for Tilapia surimi gel when heating temperature increase due to tyrosine residue were buried in a hydrophobic environment during protein denaturation.

3.2.4. Conformation of hydrophobic interactions

The band in the 2800-3050 cm^{-1} region of Raman spectrum is assigned to C-H stretching vibrations and bending in aliphatic amino acid [3]. Fig. 5 shows the intensity values of strongest C-H band in the range 2930-2933 cm^{-1}.

In surimi raw, the intensity value of strongest C-H band located near 2930 cm^{-1}. These bands have a trend of shifting to high wavenumber when the heating temperature increased from 30 to 70 ^{o}C. However, the wavenumber shift degree from 2930 cm^{-1} to 2932 cm^{-1} was low. The unfolding of protein during the heat-induced gelation may lead to the exposure of methyl of methylene, which resulted in the wavenumber shifts [6]. These band intensity decrease with

increase of heating temperature from 30 to 50 °C, however, with the temperature further increasing, the intensity of this band increased. Bouraoui et al. also [1] reported this band in cooked surimi shifted lightly to higher wavenumber compared with raw material. The change in location is assigned to the vibrations of the C-H stretching band demonstrate changes in the environment of aliphatic C-H group which may be related to hydrophobic interaction [7].

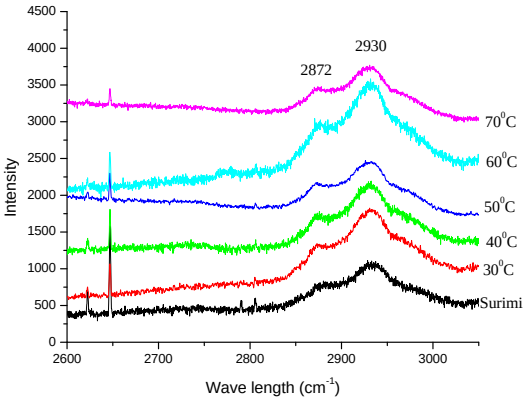

Figure 5: Raman spectra in the 2600-3050 cm^{-1} region of Tilapia surimi at different incubation temperature.

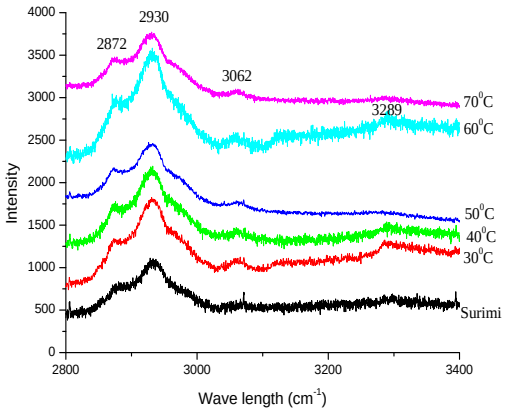

Figure 6: Raman spectra in the 2800-3400 cm^{-1} region of Tilapia surimi at different incubation temperature.

3.2.5. Changes of C-H stretching vibrations in the 2800-3400 cm^{-1} region

Hydrophobic groups of amino acids, peptides, and proteins exhibit C-H stretching vibrational bands in the 2800-3400 cm^{-1} region. Bands found near 2874-2879 cm^{-1} are assigned to CH_3 symmetrical stretching and R_3C-H stretching bands of aliphatic amino acids, whereas the C-H stretching bands of aromatic amino acids can be found near 3061-3068 cm^{-1} [1].

The band at 3289 cm^{-1}, which reflects the OH stretch, indicated a lower intensity for all sample (Fig. 6). This probably suggested that heated surimi underwent a higher rate of dehydration because of protein-protein interactions through aggregation mechanisms during heating process.

4. CONCLUSIONS

Gel formation in Tilapia surimi involves partial denaturation of protein followed by irreversible aggregation which results in a three dimensional network. During the heat induced gelation of protein, the secondary and tertiary structure of protein, molecular environment and textural changes were taken place. There was a decrease in α-helical structure accompanied by an increase in random coil structure (at 30-50 °C) and ß-sheet (over 60 °C). The changes of secondary structure such as disulfide bond reaction (30-40 °C) and hydrophobic interaction (60-70 °C) as well were occurred. Texture property changes were also determined by texture analysis. All these features contribute to the formation strong, irreversible heat-induced gel especially at 30-40 °C of incubation temperature.

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TÓM TẮT

NGHIÊN CỨU SỰ BIẾN ĐỔI CẤU TRÚC PROTEIN CỦA SURIMI CÁ RÔ PHI TRONG QUÁ TRÌNH TẠO GEL BẰNG PHỔ RAMAN

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Biến đổi cấu trúc protein và đặc tính cấu trúc của surimi cá rô phi trong quá trình tạo gel được xác định bằng phổ Raman. Sự thay đổi vùng amide I (1600-1700 cm^{-1}) cho thấy khi gia nhiệt cấu trúc xoắn α giảm đồng thời cấu trúc gấp nếp β và cuộn xoắn ngẫu nhiên tăng. Sự tạo thành liên kết cầu disunfua được quan sát ở dải băng tại 500-60 cm^{-1} với các mẫu khi ủ ở nhiệt độ 30-40 ^oC và sự hình thành liên kết này cho cấu trúc gel surimi có độ bền chắc cao. Cường độ dải băng tại 758 cm^{-1}cũng như sự giảm nhẹ tỉ số cường độ cặp đôi tyrosin I853/I826 khi nhiệt độ tăng lên 60-70 ^oC cho thấy tương tác kị nước tham gia vào quá trình tạo gel bởi nhiệt ở surimi cá rô phi.

Từ khóa: surimi cá rô phi, cấu trúc protein, phổ Raman.