IMAGING OF FAT CRYSTALS WITHIN PORK ADIPODE TISSUE

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Abstract – Crystalline states of fats such as crystallinity and the type of crystal polymorph are the factors deciding physical properties of fat based food. To reveal the crystalline states of meat fat, porcine adipose tissue was investigated by Raman microscope. After 2-month incubation in a refrigerator (3.8 ± 2.5°C), fat crystallinity within the tissue was clearly increased. Also, a change in the distribution pattern of β'-polymorph has been observed. These changes suggest that a more stable crystalline state is developed after the incubation treatment. Raman spectroscopic technique can reveal the crystalline states within meat adipose tissues at a microscopic scale.

Key Words – Fat crystallinity, Raman microspectroscopy, β'-polymorph

- INTRODUCTION

It is well known that crystalline states of fats affect decisively on physical properties such as mechanical strength and melting point of fat-based food. In the meat industry, however, the importance of the crystalline states of fat within meat tissue has not been fully recognized.

Recently, a Raman spectroscopic technique has been developed to investigate the fat crystalline states within pork adipose tissues.[1] This technique can measure fat crystallinity as well as type and content of a key crystal polymorph, β'-polymorph.

Fats exhibit crystal polymorphism and form several types of polymorph. Among them, β'-polymorph forms finely dispersed crystals, which are prone to grow together with adjacent crystals and contribute to form crystal networks.[2] Crystal networks decide the macroscopic mechanical strength of the fat system.[3] It is therefore worth trying to acquire the image of this polymorph.
In the present study, we extend the Raman technique to samples under microscope, to reveal the crystalline states of fats within pork adipose tissues at a microscopic scale.

- MATERIALS AND METHODS

Sample Tissue

Subcutaneous adipose tissue was acquired from back fat (lumber position) of a female pig (live weight of 93.4 kg) which was a cross of a Landrace × Large White sow and a Duroc boar. Inner layer (approximately 1-cm thick) of the subcutaneous adipose tissue was sampled, and kept in a refrigerator (3.8 ± 2.5°C) for a day according as pork production practice. Then, about 4 cm × 4 cm area of the layer was cut into pieces, sealed in small bags (polyethylene, film thickness 0.04 mm) and stored at −20°C until experiments.

Sample Preparation and Raman Micro-spectroscopic Measurement

Two months before Raman microspectroscopic measurement, a few sample pieces were transferred to the refrigerator and started to incubate at 3.8 ± 2.5°C. Rest pieces were transferred to the refrigerator just before Raman measurement and they were not experienced the incubation.

In the refrigerator, the pieces of adipose tissue were sliced in approximately 0.5-mm thin using a razor. The sample slices were put on a metal block whose temperature was kept at 0°C during the Raman spectroscopic measurement, and covered with a glass cover slip. Their Raman spectra were acquired using a lab made 532-nm excitation Raman microspectroscopic system with the laser focal point size of 8-μm in diameter and 45-μm in the parallel direction with the optical axis. Excitation laser power was 5 mW and the signal accumulation time was 10 s for one spectrum. A spectrum was obtained every 4-μm step along with X and Y axis of microscopic view and a set of

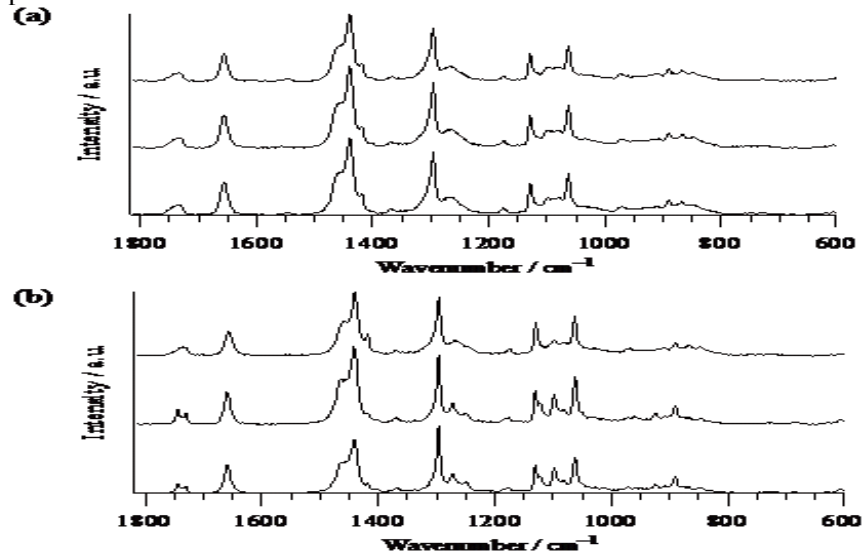

Figure 1 Some typical Raman spectra of the samples. (a) From the sample without incubation, (b) from the sample after 2-month incubation in the refrigerator (3.8 ± 2.5°C). Three spectra were acquired from different pixels of a microscopic view.

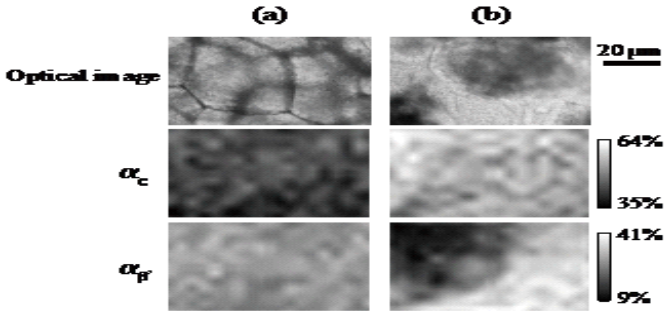

Figure 2 Images of α_C (crystallinity) and $\alpha_{\beta'}$ (content of β'-polymorph) of each optical image. (a) From the sample without incubation, (b) from the sample after 2-month incubation in the refrigera

Raman spectra for one view was acquired within 40 min.

Spectrometric Analysis of Crystalline State

Crystallinity and the content of β'-polymorph were calculated from obtained Raman spectra by using the equations reported previously.[1] Crystallinity, or the fraction in percentage of the crystalline phase, was denoted by α_C, and the content of β'-polymorph in the total fat was

denoted by $\alpha_{\beta\prime}$.

(1)

(2)

where *I* denotes the integrated intensity of the Raman band at the wavenumber identified by a subscript. The integrated intensity of a band was determined by a curve-fitting procedure applied to the spectral region containing bands which significantly influence the intensity of the target band. Since the number and the shape of these bands were not known, a minimum number of bands and the Lorentzian shape were assumed.

- RESULTS AND DISCUSSION

Some typical Raman spectra of the samples are shown in Figure 1. One spectrum corresponds a specific pixel of a Raman microscopic view. From every spectrum within a view, crystallinity (α_C) was calculated by using equation (1) and the image of α_C was built (Figure 2).

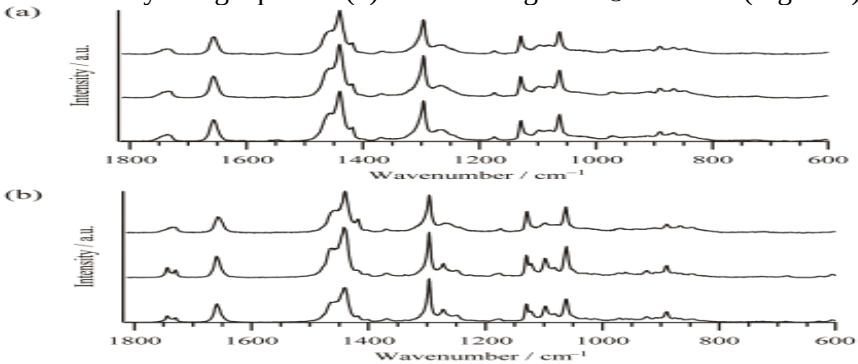

Figure 1 Some typical Raman spectra of the samples. (a) From the sample without incubation, (b) from the sample after 2-month incubation in the refrigerator (3.8 ± 2.5˚C). Three spectra were acquired from different pixels of a microscopic view.

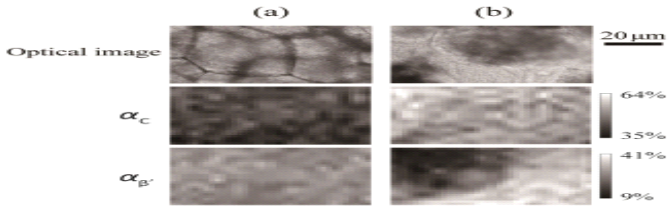

Figure 2 Images of α_c (crystallinity) and $\alpha_{\beta'}$ (content of β'-polymorph) of each optical image. (a) The sample without incubation, (b) the sample after 2-month incubation in the refrigerator.

Within the view of the sample without incubation (Figure 2(a)), α_C ranges between 35% and 51% and its average value was 43%. Authors previously shown that α_C of pork carcass back fat after 24-h cooling at 4.3°C was about 45% by using macro Raman spectrometer,[1] and this value is coincide with the average α_C value of the present study. By this study, α_C distribution at the microscopic scale has been revealed and unevenness (patchy pattern) is detected at the special resolution of the microscope. It means there are crystals/crystal aggregates whose sizes are larger than the special resolution.

After 2-month incubation in the refrigerator, the image exhibits higher α_C intensity (average 54%). Since crystallization is a kinetic process, time factor greatly affects on development of fat crystals and it is reasonable that the crystallinity become higher after 2 months. Raman

microscope can reveal how fat crystals develop within meat adipose tissues.
The sample exhibits a typical Raman band of β'-polymorph at 1418 cm^{-1} in the both samples (Figure 1). The content of β'-polymorph ($\alpha_{\beta'}$) is calculating using the intensity of this band (equation (2)).[1]
A large difference is observed in $\alpha_{\beta'}$ distribution pattern between the two samples (Figure 2). Average value of $\alpha_{\beta'}$ of the view is the same for both samples (27%), however, $\alpha_{\beta'}$ is ranging more widely for the 2 months incubated sample (10 ~ 41%) compare to the sample without incubation (22 ~ 34%). The images clearly show that β' -polymorphs become to be gathered in one place (lower right area of Figure 2(b), $\alpha_{\beta'}$) after 2-month incubation in the refrigerator.

The area where the content of β'-polymorph is low (upper left area of the image) shows high crystallinity (Figure 2(b), α_C). It means that there are crystals other than β'-polymorph. β is the highest possible polymorph since this polymorph is the most stable one, and less stable polymorphs generally recrystallize into β according to the law of thermodynamics.[4] It is likely that this polymorphic transition significantly proceeds within meat tissue after 2-month incubation in the refrigerator. It is known that recrystallization involving polymorphic transition to the β-polymorph tends to lead to a decrease in mechanical strength, often to a considerable extent.[4] Also, the change in β'-polymorph distribution pattern possibly leads to some changes in the structure of crystal network and thereby modifies mechanical strength. We are now studying the effect of such changes in fat crystalline states on the physical properties of meat.

- CONCLUSION

Images of crystalline states of fat within meat tissue were obtained by using Raman microscope. Crystallinity and the content of β'-polymorph can be visualized, and changes in these parameters are observed after 2-month incubation in the refrigerator. It seems to be due to the development of a more stable crystalline state. Raman microspectroscopy is a plausible technique to investigate microscopic crystalline states of fat, and this technique will serve basic knowledge to understand physical properties of meat adipose tissues.

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