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In Situ Raman Spectrometric Analysis of Crystallinity and Crystal Polymorphism of Fat in Porcine Adipose Tissue

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ABSTRACT: From the adipose tissues of pork carcasses stored in a refrigerator, Raman spectra were observed in situ by a portable Raman spectrometer. The observed Raman spectra, which were almost completely due to fat, showed clear dependence on the refrigeration time and carcass temperature. This dependence reflected an increase in the crystallinity of the fat and a change in the fraction of the β' polymorph. Evidence of changes in the packing order of the aliphatic chains of acylglycerol molecules was obtained, and the changes lasted for a long time after the temperature reached the lowest point (4.3 °C). Possibilities of using Raman spectrometry as a tool for routine monitoring of the conditions of carcasses as well as for research on the improvement of the mechanical strength of the adipose tissue are discussed.

KEYWORDS: *adipose tissue, crystallinity, fat, in situ Raman spectrometry, meat quality, polymorphism, pork carcass, refrigeration, acylglycerol*

INTRODUCTION

Fat in foods consists of acylglycerol in the crystalline, liquid crystalline, and melt states. The crystalline states strongly affect the quality of fat-based foods. Especially in industries producing butter, margarine, shortening, and other confectionary fats, the crystalline states have been extensively studied in relation to mechanical strength of the products.[1−4] In the meat industry, however, the importance of the crystalline states of fat in meat has not been fully recognized. To the best of our knowledge, little is known about the crystalline states of fat in adipose tissue, although some researchers have studied the states of extracted fat.[5,6] The mechanical strength of meat adipose tissue is the important characteristic relevant to the handling and processing of the meat. Pork carcasses with excessively soft adipose tissues are graded as "substandard", and they are sold at low prices.[7,8]

The mechanical strength of a fat system is related to its structures at different levels, namely, the crystal structure (atomic and molecular arrangements in crystal), clusters of crystals, and the network of clusters.[1−4] In this size-ascending order they form a structural hierarchy, and the crystal network finally decides the macroscopic mechanical strength of the fat system.[3,4] These structures are greatly influenced by temperature as well as by the molecular structure of acylglycerol, crystallinity, and crystal polymorphism.[3,4]

An acylglycerol molecule has one to three aliphatic chains composed of the methylene (CH_2) units, and the structures of the chains affect the compatibility of chain packing on crystallization. The packing compatibility brings about a variety of crystal structures, namely, polymorphs. The crystal polymorphs are roughly classified as α (hexagonal), β' (orthorhombic), and β (triclinic) according to the types of chain packing within a crystal subcell.[1,9] The packing compatibility also affects the crystallinity of the system by modifying the chemical potential of crystals.[10] Crystallinity and crystal polymorphism determine not only the shape, size, and number of crystals but also the formation of crystal clusters and cluster network.[2,4] These structural factors should be adequately controlled to improve the mechanical strength of a fat system.

From a practical viewpoint, it is desirable to evaluate these factors on the site of food production. Analytical methods such as chromatography, nuclear magnetic resonance, X-ray diffraction, and calorimetry are widely used to study various aspects of fat structures,[11,12] but they are not particularly suitable for in situ analysis. Magnetic resonance imaging (MRI) might be an informative technique to obtain information on the crystal states of fat within foods; however, it is not realistic to introduce an MRI instrument to a food production line. Meanwhile, compact Raman spectrometers recently developed are attractive for this purpose. Raman spectrometry is an analytical method that enables noncontact and noninvasive (if care is taken) examinations of target objects, and furthermore, it is sensitive to fat structures.

An aliphatic chain gives strong Raman scattering, which reflects sensitively changes in the chain structure. It has been shown that the molecular structure of acylglycerol, one of the factors affecting mechanical strength, can be analyzed by Raman spectrometry.[13,14] Also, it has been shown that Raman spectroscopy can provide information on the molecular order of aliphatic chains in the solid and liquid states[15−18] as well as the type of crystal polymorphs of fats.[15,19−21]

The present paper reports an in situ Raman spectrometric analysis of fat in porcine adipose tissue. The usefulness of the Raman indicators for evaluating the crystallinity and crystal polymorphism of food fat is discussed.

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■ MATERIALS AND METHODS

Pork Carcass Preparation and Cooling. The present study, which involves animals, was approved by NARO (Approval 11120501) and conducted in accordance with international guiding principles.[22]

Six barrows from the same litter, which were crosses of a Landrace × Large White sow and a Duroc boar (the typical commercial crossbred), were fed by using the same multiphase feeding regimen with feeds of general commercial formula until their live weights became 109.5 ± 5.2 (mean ± SD) kg. Slaughtering was conducted on two days with an interval of one week between them. The room temperature of the slaughterhouse was about 7 °C. The carcasses were skinned, split into two sides, and rinsed with warm water at about 30 °C. After the dressing, weights of the carcasses were 69.7 ± 3.1 kg, which were in the range for standard pork carcasses.[7,8] At 20 min after slaughter, the carcasses were transferred into a refrigerator (44 m^3) equipped with an air conditioner of cooling capacity 2700 kcal h^{-1} (ERA-22C1, Mitsubishi Electric Co., Tokyo, Japan). A conventional chilling procedure[23] was applied: The temperature and relative humidity in the refrigerator were set, respectively, at 4 °C and >90% throughout the experiment. The air circulation rate was 80 times the refrigerator volume per hour, and the wind velocity measured by an anemometer (405-V1, Testo K.K., Yokohama, Japan) was about 0.7 m s^{-1} at the carcass surfaces.

Temperatures at the surface and the deep parts (lumbar longissimus muscles) of the carcass shown in Figure 1 and the room temperature

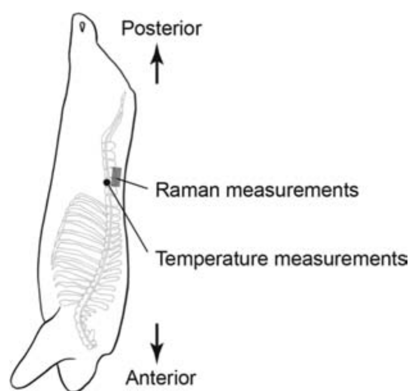

Figure 1. Positions on a hanging pork carcass chosen for measuring temperature and Raman spectra.

were measured by a thermal data acquisition system (E830, Tateyama Kagaku Industry, Toyama, Japan) equipped with calibrated T-type thermocouples. Because the cooling process of the carcass surface seems to follow Newton's law of cooling,[24] the temperature T at the surface is expressed as

$$T_t = (T_0 - T_f)\, e^{-ct} + T_f \qquad (1)$$

where t denotes the cooling hours in the refrigerator, c is a constant determined by the heat capacity, area, and heat-transfer coefficient of the carcass surface contact with air flow, and T_t, T_0, and T_f correspond, respectively, to temperatures at time t, time 0 (at the beginning of cooling), and time f (at the final stage). T_f is equal to the air temperature in the refrigerator.

The rate of temperature change, which is obtained by differentiating eq 1 with respect to t, is given as

$$dT_t/dt = -c(T_0 - T_f)\, e^{-ct} \qquad (2)$$

Raman Spectrometric Measurements. Raman spectra were measured from the carcass surfaces (the outer layer subcutaneous adipose tissues) at 5−8 cm distances from the mid-dorsal axis at the third−fourth lumbar vertebrae (Figure 1) by using a portable Raman spectrometer (EZRaman-I, Enwave Optronics, Irvine, CA, USA) at a spectral resolution of 7.1 cm^{-1}. The spectrometer was equipped with an optical fiber probe with an objective lens (focal length = 6 mm, N.A. 0.3) at its end. A 785 nm laser beam (150 mW) for Raman excitation was defocused on the carcass surface, and backscattered Raman signals were collected and accumulated for 60 s.

Raman spectra were observed from three different points within the designated area of the outer layer subcutaneous adipose tissue (Figure 1) at each time of measurement. Such measurements were made of three carcasses for the entire period of measurements and of the other three carcasses for the first four days after transferring the carcasses into the refrigerator. The measurements were conducted in the refrigerator intermittently for 11 days after the carcasses had been transferred there. The observed Raman spectra were processed in a way to be described later.

In the observed Raman spectra of the adipose tissue, the Raman bands of fat should be discerned from bands that may arise from other components. For this purpose, Raman spectra were observed from extracted fat without connective tissue. The sample of fat for this measurement was prepared in the following way. A portion of porcine subcutaneous adipose tissue was cut into small pieces (approximately 0.5 mm cubes), put in a glass test tube with a screw cap, and heated up to 80 °C in a water bath. After tens of minutes, exuded oil was collected and used without further purification. Raman spectra from this sample were observed in the laboratory. The fat was thoroughly melted at 80 °C, and a small amount of the melt (about 10 μL) was dropped on a CaF_2 coverslip (0.3 mm thick) placed on a temperature-controlled stage (Linkam 10021, Tadworth, Surrey, UK) set at 30 °C. The 785 nm excited Raman spectra of the extracted fat were observed with a spectrometer described elsewhere[21] with a backscattering geometry at a spectral resolution of 3.8 cm^{-1}. With a laser beam of 100 mW power, a Raman spectrum was obtained for an exposure time of 10 s, and the results of two measurements were added.

Handling of Observed Raman Spectra. On the basis of a comparison among the spectra, cosmic-ray lines existing in as-observed Raman spectra were removed. A background spectrum taken in a dark condition was also subtracted from as-observed Raman spectra to eliminate signals from the CCD used and to obtain only the spectra due to signals from samples. All three Raman spectra observed at about the same time after transfer of the carcass into the refrigerator were averaged. All of the Raman spectra of the adipose tissue shown later were obtained in this way. The integrated intensity of a band was determined by a curve-fitting procedure applied to the spectral region containing bands that significantly influence the intensity of the target band. Because the number and shape of these bands were not known, a minimum number of bands and the Lorentzian shape were assumed. The baseline of the spectral region under study seemed to have a tilt, probably due to fluorescence. To account for this tilt, a linear function or a Lorentzian function with a very broad bandwidth (half-width at half-maximum > 500 cm^{-1}) was used to the spectral region under study. The procedure was conducted without restriction to the fitting parameters because they could change with the physical states of samples. It was confirmed that the bandwidth parameters were larger than the spectral resolution of the Raman spectrometer used in the present study.

Statistical Analysis. Comparisons among the data obtained at different times of measurement were made by the Tukey−Kramer test at 5% significance using the GLM procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC, USA). The carcass number (nested within the slaughter date), slaughter date, and cooling time were assigned to the variables of the statistical model. Before the analysis, the normality of the data was validated by the UNIVARIATE procedure of SAS, and the equality of variance of the data was ensured by the Box−Cox transformation using the TRANSREG procedure of SAS.

■ RESULTS AND DISCUSSION

Sample Temperature. The temperature and the rate of temperature change influence decisively the crystallinity and crystal polymorphism of fat to be discussed later in this paper. Just before the pork carcasses were transferred into the refrigerator, the surface temperature was about 24 °C (Figure 2). After the transfer, it sharply decreased to 16 °C within the

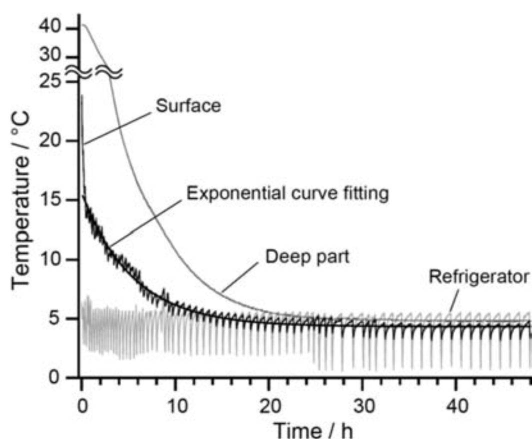

Figure 2. Temperatures at the surface and deep part of a carcass and in the refrigerator. The result of exponential curve fitting for the surface temperature is also shown.

first 15 min at a maximum cooling rate of −2 °C min^{-1}. Subsequent temperature changes could be expressed accurately by eq 1, excluding the influence of temperature fluctuation (Figure 2) due to the on−off thermostat function of the refrigerator, which is commonly observed in meat-producing plants, and the carcass surface temperature T_t at time t was given as

$$T_t = (16.2 - 4.3)\,e^{-0.175t} + 4.3 \quad (SD = \pm 0.4)$$

The rate of temperature change was determined from eq 2 as

$$dT_t/dt = -2.08\,e^{-0.175t}$$

Sample temperatures and cooling rates given later in this paper are the values derived from the above equations. It was confirmed that the temperature of the deep parts of carcasses adequately decreased in fulfillment of the general requirement for pork production (>10 °C at 10 h and <15 °C at 18 h) (Figure 2).

Observed Raman Spectra. Whenever biological tissues are studied by Raman spectroscopy, fluorescence from unknown origins in the samples can be a serious problem, because it is often much stronger than Raman scattered light, which is generally weak.

In Figure 3, the 785 nm excited Raman spectrum of the adipose tissue and that of the extracted fat are compared. The spectrum of the adipose tissue in Figure 3a has a baseline rise over a wide spectral region probably due to fluorescence, but the spectrum clearly shows many bands that are identified as the Raman bands of fat in comparison with the spectrum of the extracted fat shown in Figure 3b. It is noted that bands observed from the adipose tissue are all due to fat and not to any other chemical species existing in the adipose tissue, although porcine subcutaneous adipose tissue contains 76.3% fat, 18.3% water, and 5.1% proteins.[25] Water is known to give a weak, broad Raman band around 1600 cm^{-1}, but it is not seen in Figure 3a. Proteins show various Raman bands, but none of them are found in Figure 3a because of their small contents in the adipose tissue.

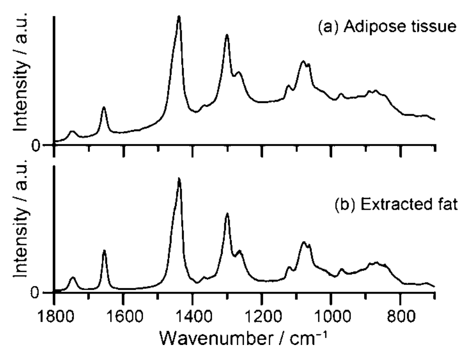

Figure 3. 785 nm excited Raman spectra of (a) porcine subcutaneous adipose tissue and (b) extracted pork fat.

In Figure 4, the Raman spectra of the adipose tissue observed at various times after transfer of the carcasses into the refrigerator are shown. From these spectra, time- and temperature-dependent changes in the crystalline states of the fat in the adipose tissue can be elucidated as described below.

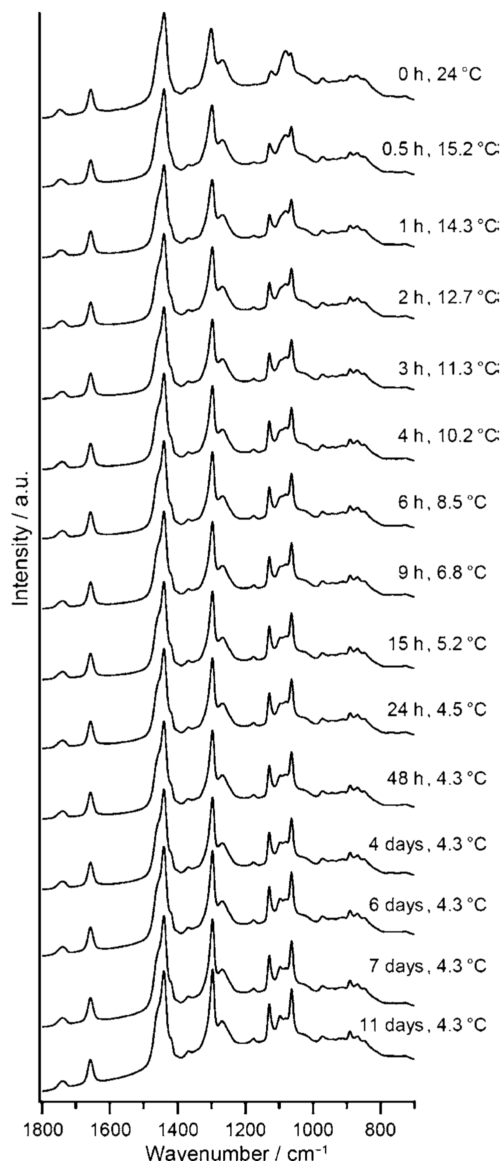

Figure 4. Time and temperature dependence of the Raman spectra of subcutaneous adipose tissue.

Crystallinity. In Figure 4, the Raman bands in the region below 1600 cm^{-1} are considered to arise from the polymethylene chains of fat. Time-dependent spectral changes

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consist mostly of sharpening of bands and small shifts of peak positions, suggesting that these spectral changes are associated with intermolecular effects or, in more concrete terms, the crystallization process of the polymethylene moieties in the adipose tissue.

The crystalline states of polymethylene systems have been a subject of abiding interest for many years. Strobl and his co-workers applied Raman spectrometry to the study of this subject and discussed methods for evaluating the fractions of various phases including the crystalline phase.[17,18]

We applied a method proposed by Strobl's group to evaluate the crystallinity of the fat in the adipose tissue. The method uses the intensities of the Raman bands due to the CH_2 twisting vibration. Representative Raman spectra of the fat in the adipose tissue in the CH_2 twisting region are shown in Figure 5.

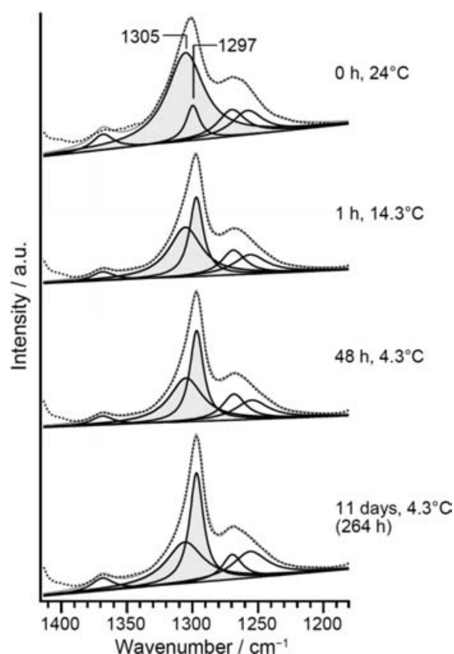

Figure 5. Representative Raman spectra of the adipose tissue in the region of 1400−1200 cm^{-1}, with the bands at 1305 and 1297 cm^{-1}. Decomposed bands are shown in full curve, and the synthesized spectrum is shown in a gray curve. Observed spectral data are indicated in a dotted curve.

A band decomposition procedure was applied to the Raman spectra, and the main component bands at 1305 and 1297 cm^{-1}, the intensities of which greatly change with time after the sample was stored in the refrigerator, were identified as being due to the melt and crystalline phases, respectively.

In the Raman spectra of polyethylene,[17,18] bands corresponding to the above two bands at 1305 and 1297 cm^{-1} were found, respectively, at 1303 (or 1305) and 1295 cm^{-1}, and the sum of the integrated intensities of these two bands was found to be independent of temperature. Because of this constancy, the total intensity of the two bands was adopted as an internal intensity standard for deriving the crystallinity.[17,18]

On the basis of the work of Strobl's group outlined above, the crystallinity, or the fraction in percentage of the crystalline phase of the fat in the adipose tissue (α_C), may be evaluated by the equation

$$\alpha_C = \frac{I_{1297}}{I_{1297} + I_{1305}} \times 100 \quad (3)$$

where I denotes the integrated intensity of the Raman band at the wavenumber identified by a subscript. Essentially the same indicator was used to evaluate the crystallinity of polymethylene systems such as *n*-alkanes,[26] polyethylene,[27] and fatty acids.[28]

In Figure 6a, the values of α_C calculated from the Raman spectra in Figure 4 are plotted against time and temperature.

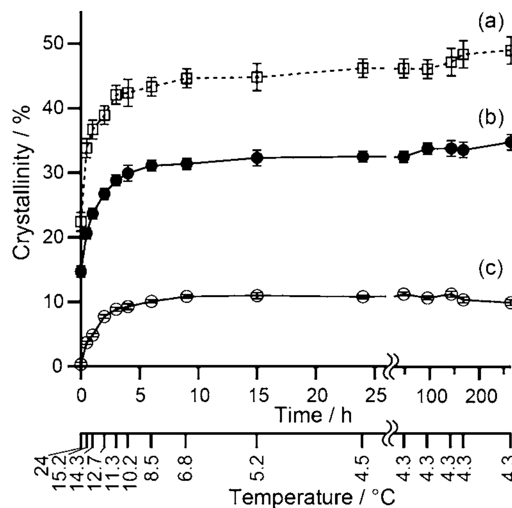

Figure 6. Changes in crystallinity and fraction of β' polymorph in the fat of the adipose tissue: (a) α_C; (b) C_{trans}; (c) $\alpha_{\beta'}$. Points represent the least-squares means, and bars indicate standard errors.

The crystallinity increased rapidly during the first 24 h and reached 46%. Meanwhile, the temperature on the carcass surface decreased to 4.5 °C with cooling rates ranging from −2 °C min^{-1} to −0.03 °C h^{-1}.

In addition to α_C described above, another indicator was more recently proposed by Brambilla and Zerbi in a study on local order in liquid *n*-alkanes.[29] This indicator, which is denoted by C_{trans}, uses the intensity of the Raman band at 1130 cm^{-1}, which arises from the in-phase C−C stretching vibration when a polymethylene chain has a long sequence of the C−C bonds in the *all-trans* planar conformation. Because the aliphatic chains of an acylglycerol molecule have the *all-trans* planar sequences in crystal, it is reasonable to use this indicator for evaluating the crystallinity of fats. This indicator is expressed as

$$C_{trans} = \frac{(1.3)I_{1130}}{I_{1297} + I_{1305}} \times 100 \quad (4)$$

The factor 1.3 in the numerator on the right-hand side of this equation is an experimental correction coefficient introduced by Brambilla and Zerbi for the contribution of each CH_2 group in the *all-trans* planar sequence to I_{1130} relative to $(I_{1297} + I_{1305})$ in the denominator, which represents the CH_2 twisting intensity arising from all of the CH_2 groups.

The value of C_{trans} obtained from each Raman spectrum in Figure 4 is plotted in Figure 6b, which shows a rapid increase with time and reaches 33% during the first 24 h. The value of 33% is close to the crystallinity of lard reported previously (∼30% at 5 °C with a cooling rate of −0.1 °C min^{-1}) by a study using nuclear magnetic resonance.[5]

Although the values of α_C and C_{trans} show similar dependencies on time (and temperature), a considerable difference is seen between the crystallinity ultimately reached: 48% for α_C and 35% for C_{trans}. The difference of 13% can be attributed to the facts that (1) what are counted by the two indicators may not be exactly the same and (2) the correction coefficient for I_{1130} in eq 4, which was derived from the analysis of the Raman spectra of liquid *n*-alkanes, may deviate from 1.3 in the Raman spectra of the fat. According to Strobl's group,[17] α_C includes a part called the transition region adjacent to the crystallites, although the molecular structure of polyethylene in this region is not clear. This may account for the result that α_C is always larger than C_{trans}. Which of the results from the two methods described above is more reliable is a matter to be studied further, although, as mentioned above, C_{trans} gave an estimate in agreement with the solid fat content acquired by NMR method.

In concluding this section, it would be safe to state that one-third to half of the fat in the adipose tissue is in the crystalline states when the adipose tissue is adequately cooled to 4.3 °C by conventional refrigeration procedures. According to previous studies on Raman spectra of fats,[15,16,19,20] spectral regions other than those used in this study are also informative in the study of crystallinity. In the ester carbonyl C=O stretching region, a band due to fats in the liquid phase and bands arising from those in the solid phase exist at 1749 and 1746−1731 cm^{-1}, respectively.[15] The difference in wavenumber is attributed to constraints imposed on the ester carbonyl region by intra-molecular and intermolecular interactions, which are prominent in the solid phase.[30,31] The intensity ratio of these bands may be useful as a measure of the crystallinity of fats. However, because these bands have relatively weak intensities (Figure 4), a longer time is needed to measure a spectrum with an adequate signal to noise (S/N) ratio. It has been shown that the C−H stretching region (3000−2700 cm^{-1}) has information on lateral interactions between polymethylene chains in fats.[15] This spectral region was not included in the present study, because it was difficult to obtain Raman spectra with high S/N ratios by using the 785 nm laser light for Raman excitation.

Crystal Polymorphism. As mentioned in the Introduction, acylglycerol has crystal polymorphism. It is known that the β' polymorph forms finely dispersed crystals, which are prone to grow together with adjacent crystals and contribute to form crystal networks, thereby giving firmness to the fat system.[2] It is therefore significant to measure the fraction of this polymorph.

According to Strobl's group,[17,18] it is possible to derive the fraction of the β' polymorph from the intensity of the Raman band at 1418 cm^{-1} (1416 cm^{-1} in ref 17), which is characteristic of the β' polymorph of fats.[32,33] A few representative Raman spectra in the region of this band are shown in Figure 7, together with the results of band decomposition. The fraction in percentage of the β' polymorph (denoted by $\alpha_{\beta'}$ instead of α_c in ref 17) can be calculated by the following equation, which is the same as eq 4 in ref 17, but is expressed in a simpler manner.

$$\alpha_{\beta'} = \left(\frac{1}{0.493}\right)\frac{I_{1418}}{(I_{1297} + I_{1305})} \times 100 \quad (5)$$

The factor of (1/0.493) is the experimentally acquired intensity correction coefficient for the 1418 cm^{-1} band relative to (I_{1297} + I_{1305}).[17,18]

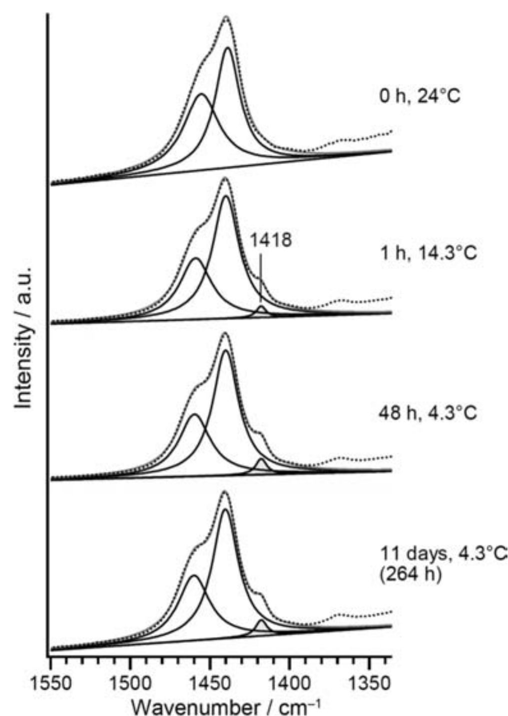

Figure 7. Representative Raman spectra of adipose tissue in the region of 1550−1350 cm^{-1}, with the band at 1418 cm^{-1}. Decomposed bands are shown in full curves, and the synthesized spectrum is shown in a gray curve. Observed spectral data are indicated in a dotted curve.

The values of $\alpha_{\beta'}$ calculated from the Raman spectra in Figure 4 are given in Figure 6c. With the steep decrease in temperature in the initial stage of measurements, $\alpha_{\beta'}$ rapidly increased to 11% and then decreased slightly. It should be noted here that the β' polymorph actually consists of two forms (β'_1 and β'_2) and that the intrinsic contribution of the β'_2 form to the 1418 cm^{-1} band may be weaker than what is expressed by the intensity correction factor of (1/0.493) because of the imperfect orthorhombic structure of this form.[6,33] It is therefore possible that eq 5 underestimates the β' fraction of the porcine fat. Nevertheless, by comparison of the estimated $\alpha_{\beta'}$ values with the C_{trans} values obtained by eq 4, it can be said that the β' polymorph accounts for roughly one-third of the total crystalline phase.

Campos et al. reported that, when lard was crystallized rapidly, the polymorph observed was mostly β'; in contrast, when it was crystallized slowly, not only β' but also β was formed.[5] The β polymorph belongs to the triclinic system, and the polymethylene chains in this polymorph are parallel to each other.[1,9] Kalnin et al. showed that β' and β were simultaneously formed upon a slow cooling of −0.15 °C min^{-1}, and a certain amount of the α polymorph was also found upon the slow cooling.[6] In the present study, the cooling rate was slower than −0.15 °C min^{-1} during the whole period of cooling. Therefore, it seems to be highly possible that the β polymorph is the major component of the remaining two-thirds of the crystalline phase, and a certain amount of the α polymorph may also exist there.

An interesting spectral change was found in the region from 950 to 800 cm^{-1}. A few representative spectra in this region are shown in Figure 8, where the results of band decomposition are also given. The time-dependent spectral changes of the bands at 890 and 870 cm^{-1} in this figure are considered to be associated with the polymorphic transformation from β' to β and probably

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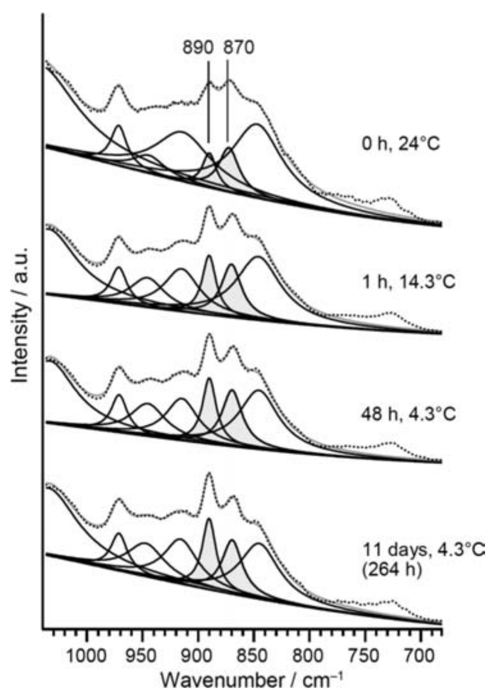

Figure 8. Representative Raman spectra of the adipose tissue, with bands at 890 and 870 cm^{-1}. Decomposed bands are shown in full curves, and the synthesized spectrum is shown in a gray curve. Observed spectral data are indicated in a dotted curve.

also with that from α to β on the grounds to be described shortly.

Both the 890 and 870 cm^{-1} bands arise from the CH_3 rocking vibration, and the difference in their positions is attributable to the conformational order at the chain ends.[34,35] The 890 cm^{-1} band arises from an extended chain having the *all-trans* conformation to its end, and it is observed for the β polymorph, the aliphatic chains of which are packed perfectly to their ends.[34,36] By contrast, the 870 cm^{-1} band, which is assignable to an aliphatic chain having the *gauche* conformation at its end,[34,35] is observed for the β' and α polymorphs, which have disorders in the chain packing at the crystal lamellar surface.[34,36] Then, the ratio of I_{890}/I_{870} may be used as an indicator of the chain-end order, which should in turn correspond to the degree of polymorphic transformation. The time-dependent change of I_{890}/I_{870} calculated from the spectra in Figure 4 is shown in Figure 9. As seen in this figure, the chain-end order continued to increase for a long time after 24 h when the temperature almost reached the lowest. This result seems to indicate that the polymorphic transformation from β' (and probably α also) to β occurs gradually even at 4.3 °C. It is known that extracted porcine fat (lard) tends to transform into the β polymorph, the most orderly and stable crystal form, because it is composed of relatively few and closely related triacylglycerol species.[37,38] It is now confirmed that this transformation occurs also in the adipose tissue.

Possible Role of in Situ Raman Analysis in Meat Production. As shown in the preceding part of the present paper, Raman spectrometry is useful for in situ analysis of the crystalline states of fat (i.e., crystallinity, crystal polymorphs, and transformations between them) in porcine adipose tissue. It is worth trying to use this method as a tool for routine monitoring of the physical conditions of meat carcasses in refrigerators.

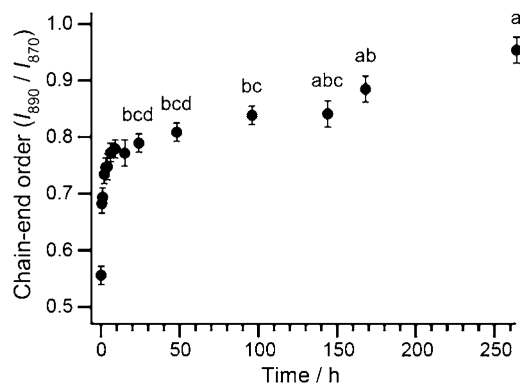

Figure 9. Changes in the packing order at the aliphatic chain ends. Points represent the least-squares means, and bars indicate standard errors. Points with different letters are statistically different ($P < 0.05$). The letters are omitted for the data taken earlier than 24 h.

Raman spectrometry has an advantage over other methods in that Raman spectral measurements can be performed with a portable instrument in a refrigerator containing meat carcasses, and observed Raman spectra can be easily processed to give useful information on the crystalline states of fat. In addition, Raman measurements are not affected by changes in physical conditions in the refrigerator such as the temperature and wind velocity. Such changes in physical conditions are common in meat-producing plants.[23,39]

There are possibilities of improving the mechanical strength of porcine adipose tissue by modifying the crystalline states of fat. The Raman spectroscopic analysis can play an important role in examining such possibilities. By applying simultaneously the previously reported Raman spectrometric method of analyzing the molecular composition of acylglycerols[13,14] and the methods described in the present study to the examination of the states of fat, a single Raman measurement may lead to the clarification of all the factors influencing the mechanical strength of fat systems. Finally, we remark that the analytical procedures used in the present study need validation in an appropriate manner. We are now preparing to validate our results at a laboratory level by using extracted fats and standard samples, with the hope of establishing a reliable method for an in situ quantitative analysis.

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Notes

The authors declare no competing financial interest.

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