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REVIEW

A Raman Spectroscopic Method of Evaluating Fat Crystalline States and Its Application in Detecting Pork Fat

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Abstract

Raman spectroscopy is one of the vibrational spectroscopies. Raman spectra of fats inform us of the vibrational states of acylglycerol molecules that reflect chemical structure, physical states, and the microenvironment at the molecular level. One of the great advantages of Raman spectroscopy is that it is less invasive; a sample can be studied *in situ* using laser light without pretreatment. Taking advantage of this spectroscopy, we investigated the crystalline states of meat fats and developed an *in situ* method of evaluating the degree of crystallinity and the crystalline polymorphs of fats. This method serves as a basis for such applications as the on-site quality measurement of meat fats and the species-specific detection of pork fat. This review describes the basics of Raman spectral analysis of the crystalline state of fat and its applications to meat products.

Discipline: Animal industry
Additional key words: crystal polymorph, fat crystallinity, halal, meat, non-destructive method

Introduction

Raman spectroscopy is one of the vibrational spectroscopies. Raman spectra inform us of the vibrational states of molecules, reflecting chemical structure, physical states, and the microenvironment at the molecular level. In meat science, Raman spectroscopy is used to determine the structure and physical states of meat components such as secondary and tertiary protein structures, hydrogen bonding between amino-acid residues, chemical composition of fats, and fat crystalline state (Beattie et al. 2008, Motoyama et al. 2013, Olsen et al. 2007, Pedersen et al. 2003, Scheier & Schmidt 2013). These structures are closely related to such important quality traits of meat as texture, nutritional quality, storage performance, and palatability.

One of the great advantages of Raman spectroscopy lies in sample pretreatment. Measurements are done without extraction, dyeing, labeling or using other contrast-enhancing agents since Raman spectra can be obtained by the application of laser light on the sample. By using Raman spectroscopy, a sample can be observed *in situ*, without modification and in a non-destructive manner.

Taking advantage of this spectroscopy, the authors have developed new methods of evaluating meat-fat crystals. This review describes the basics of Raman spectral analysis of the crystalline state of fat and its application to meat products. For a discussion of the relationship between the crystalline state of fat and meat quality, and the basic interpretations of Raman spectra of meat, please refer to other monographs (Motoyama in print, Motoyama et al. in print).

Raman spectra of meat fat

The fatty parts of meat are composed of adipose tissue. Adipose tissue contains adipocytes that are filled with

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fats and supported by loose connective tissue (Fig. 1). On average, this tissue contains 75%-80% fat (acylglycerols), 5%-20% water, and a small amount of proteins (Ministry of Education, Culture, Sports, Science and Technology 2015).

Figure 2 shows a Raman spectrum of adipose tissue from pork. The observed Raman signals mostly originated from fats, with a limited contribution from proteins and water. The large polarizability of long polymethylene-chain moieties (-$(CH_2)_n$-) of acylglycerol molecules is the fundamental source of the strong Raman signals. Bands observed below 1600 cm^{-1} in the spectrum are mostly related to those originating from the polymethylene chain. The intensity and position of the Raman bands change according to the crystalline state of the fat, as the vibrational modes reflect the microenvironment and conformation of the polymethylene chain within the crystal subcell (Motoyama 2012).

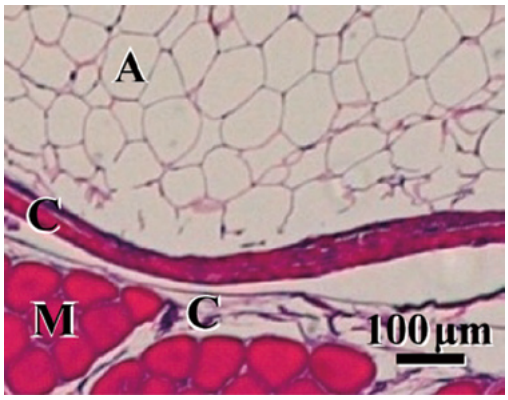

Fig. 1. A section of subcutaneous adipose tissue from a pig (5-month old female of the Landrace breed).
Adipocytes (white, about 100 µm in size, indicated by the letter A) are observed. Tissue observed at the lower side is muscle tissue (indicated by the letter M). Epimysium and perimysium connective tissues are indicated by the letter C.

On-line analysis of the crystalline states of meat fats

Once livestock animals are slaughtered and stored at chilled temperatures, the decrease in carcass temperature causes the solidification of fat. Small crystals of acylglycerols are formed within the adipocytes that join together to make a network structure. This structure provides the framework of the fat system and gives macroscopic mechanical strength to the fat (Narine & Marangoni 2005, Walstra et al. 2001). Hardness is one of the important quality characteristics of meat fats.

Time- and temperature-dependent Raman spectra of pork carcasses after slaughtering were obtained non-destructively using a portable spectrometer (Figs. 3 and 4). The skinned carcasses (69.7 ± 3.1 kg) were hoisted and then transferred to a refrigerator 20 min. after slaughtering. A conventional chilling procedure (4°C with wind velocity of 0.7 m s^{-1}) was applied and Raman spectra were measured from the carcass surface (the outer layer of subcutaneous adipose tissue) in the refrigerator. The spectrometer was equipped with an optical fiber probe with an objective lens at its end. A 785-nm laser beam (150 mW) for Raman excitation was defocused on the carcass surface, and back-scattered Raman signals were collected and accumulated for 60 s. Temperature at the surface of the carcass was also

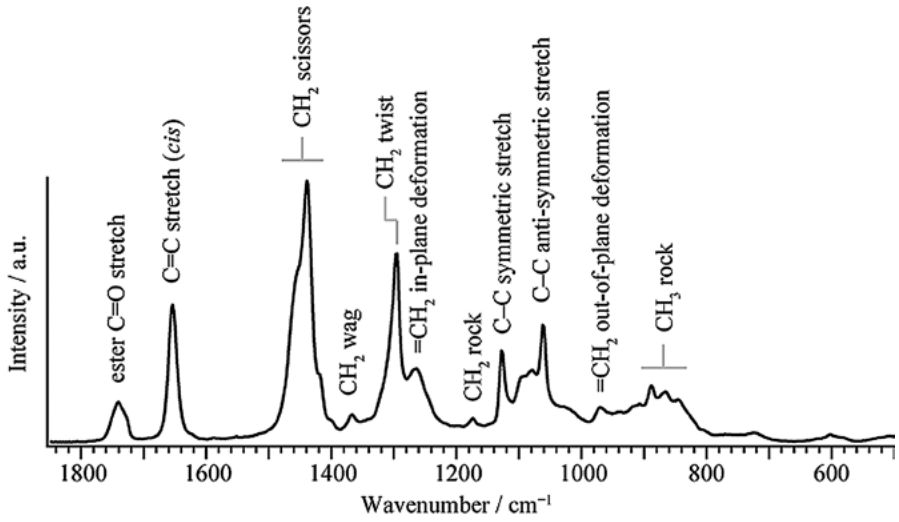

Fig. 2. A Raman spectrum of subcutaneous adipose tissue of pork (Raman excitation laser wavelength of 532 nm; laser power of 30 mW; wavenumber resolution of 5.1 cm^{-1}) and the band assignments.
Reproduced from Motoyama (in print).

measured.

The crystallinity of the fat — the percent mass of fat in the crystalline phase — can be calculated using Raman bands that originate from a polymethylene chain in the all-*trans* planar conformation (Motoyama et al. 2013). In the crystalline state, polymethylene chains are in an all-*trans* planar conformation; therefore, the intensity of the bands corresponds to the mass of the crystals. Two different Raman indices for crystallinity have been reported (Motoyama et al. 2013):

$$\alpha_C = \frac{I_{1297}}{I_{1297}+I_{1305}} \times 100$$

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

where I denotes the integrated intensity of the Raman band at the wavenumber identified by a subscript.

Index α_C uses the intensities of the Raman bands that result from the CH_2 twisting vibration (Fig. 2). Bands at 1297 and 1305 cm^{-1} were identified as originating from the crystalline and melt phases, respectively (Mutter et al. 1993, Strobl & Hagedorn 1978). The sum of the integrated intensities of these two bands ($I_{1297}+I_{1305}$) that corresponds to the mass of all the methylene groups was found to be independent of temperature (Mutter et al. 1993, Strobl & Hagedorn 1978), so the total intensity of both bands was adopted as an internal intensity standard in the denominator. Essentially, the same indicator was used to evaluate the crystallinity of such polymethylene systems as *n*-alkanes (Orendorff et al. 2002), polyethylene (Takahashi et al. 2010), and fatty acids (Kaneko et al. 1994).

Index C_{trans} uses the Raman band at 1130 cm^{-1} that arises from the C-C symmetric stretching vibration (Fig. 2) when a polymethylene chain is in an all-*trans* planar conformation. The factor of 1.3 in the numerator is an experimental correction coefficient for I_{1130} relative to the internal intensity standard ($I_{1297}+I_{1305}$) (Brambilla & Zerbi 2005). Index C_{trans} was modified as detailed later (Motoyama et al. 2016).

Figure 5 shows the changes in the values of indices α_C and C_{trans} of the pork carcasses. The values increase greatly

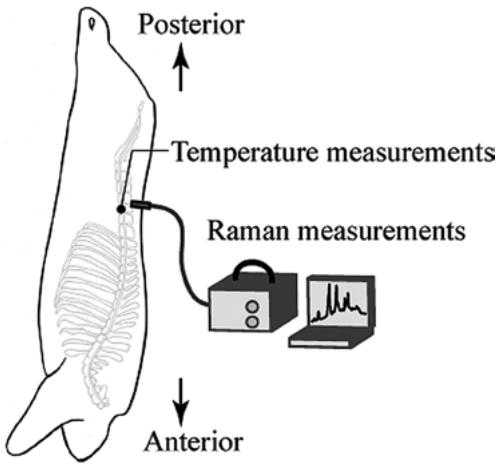

Fig. 3. Position on a hoisted pork carcass chosen for measuring temperature and Raman spectra.

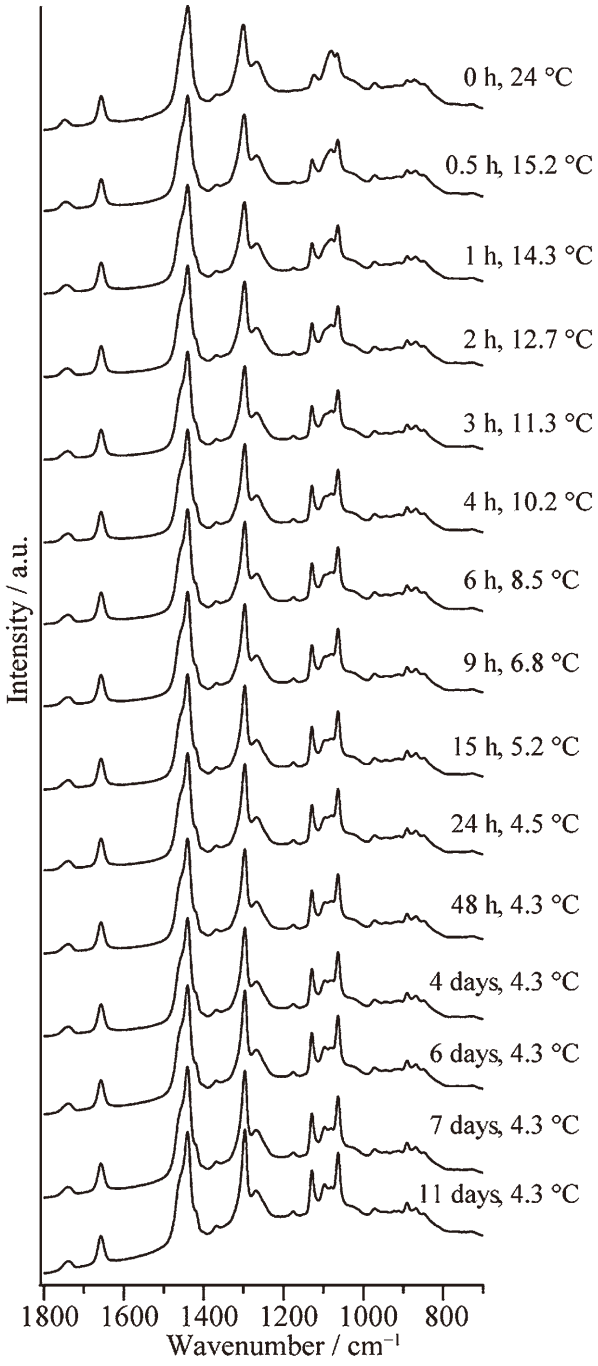

Fig. 4. Time- and temperature-dependent Raman spectra of subcutaneous adipose tissue of pork carcasses. Reproduced from Motoyama et al. (2013).

as the temperature decreases. Although the values of α_C and C_{trans} show similar dependencies on temperature and time, a considerable difference is seen between the crystallinity that is ultimately reached; 48% for α_C and 35% for C_{trans}. The difference of 13% can be attributed to the transition region adjacent to the crystallites (Mutter et al. 1993).

Fat crystals express polymorphism. The crystal polymorphs are roughly classified as α (hexagonal crystal-subcell structure), β' (orthorhombic crystal subcell), and β (triclinic crystal subcell) in ascending order of stability. Among them, the β' polymorph is related to crystal network formation and contributes to the hardness of fat-based food products (Motoyama et al. 2016, Precht 1988).

β' polymorph content can be derived from the intensity of the Raman band at 1418 cm^{-1}, which is characteristic of the orthorhombic crystal-subcell structure of the β' polymorph. The percent fraction of the β' polymorph can be calculated by the following equation (Motoyama et al. 2013).

$$\alpha_{\beta'}=\frac{1}{0.493}\times\frac{I_{1418}}{I_{1297}+I_{1305}}\times 100$$

The factor of (1/0.493) is the experimentally acquired intensity correction coefficient for the 1418-cm^{-1} band relative to the internal intensity standard ($I_{1297}+I_{1305}$) (Mutter et al. 1993, Strobl & Hagedorn 1978).

The β' polymorph increased with decreasing temperature and was 10% when the carcass temperature reached 4.3°C (Fig. 5), slightly decreasing afterward until the end of the experiment.

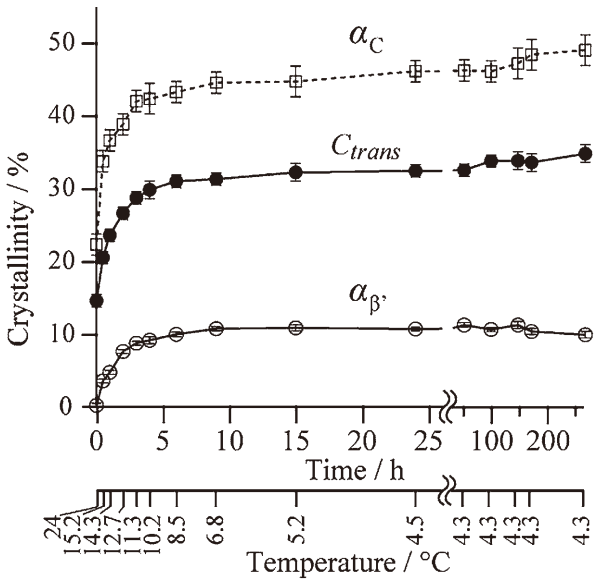

Fig. 5. Changes in Raman indices of crystallinity and fraction of β' polymorph in the fat of pork subcutaneous adipose tissue.
Points represent the least-squares means and bars indicate standard errors.
Reproduced from Motoyama et al. (2013).

Using this Raman spectroscopic method, the authors also investigated the relationship between the crystalline states of fats and the hardness of subcutaneous adipose tissue in pig carcasses on a pork production line. The hardness of the adipose tissue at the shoulder position of pig carcasses that were produced with various feeds (n=12) was clearly correlated with the Raman indices of crystallinity and the content of the β' polymorph in the fat (R^2=0.78). In view of the application of this Raman method for carcass evaluation or grading in the meat industry, the prediction ability of these Raman indices obtained from a small area of a carcass for whole carcass evaluation is now being verified on-site.

Specific detection of pork fat by Raman spectroscopy

From a food safety point of view, the fraudulent labeling of meat products threatens consumers. Adding pork meat or fat to meat products to replace more expensive meat, such as beef without adequate labeling, is one problem. A method of detecting pork may be more significant for consumers subject to allergic or religious restriction (halal or kosher).

As the acylglycerol composition differs between animal species, the crystalline state of the fat varies. Using this property, one can differentiate the animal species of fat origin (American Oil Chemists' Society 2005, Motoyama et al. 2010). By detecting the differences through Raman spectroscopy, pork fat can be specifically detected *in situ* (Motoyama et al. 2015).

When pork fats are quenched, the β'-crystal polymorph frequently forms (Campos et al. 2002, Kalnin et al. 2005). Figure 6 (a) shows optical images of the adipose tissues of pork, beef, and chicken which were covered by a glass cover slip, rapidly cooled to 0°C and then kept at this temperature. From these tissues, 785-nm excitation Raman spectra were obtained from pixel to pixel using a Raman microscope with 30-mW laser power and 30-s signal accumulation time for each spectrum. No change was observed in optical images after the measurement. From the obtained spectra, Raman spectroscopic images of the concentration of the β'-crystal polymorph relative to the total mass of crystal was constructed (Fig. 6 (b)). The concentration was calculated using the methods described in the previous section. In pork adipose tissue, the β'-polymorph exists at a higher concentration than those in beef or chicken. Pork tissue can be precisely distinguished from other meats by the discriminant *w* that contrasts the images at a certain threshold (Fig. 6 (c)).

Figure 7 shows the species-specific detection of pork adipocyte in beef-pork minced meat. The discriminant

w detected the adipocytes that were determined to be pork. Using this microscopic method, the position of the adipocyte can be investigated. Pork fats detected inside of a product means that pork was mixed during the production process, and pork fats present on the surface have probably adhered through contact after production. By using the variability of the crystalline state of fats between animal species, the authentication and quality control of meat products are possible.

Conclusions

By measuring the amount of the crystal state of fats and crystal polymorph using Raman spectroscopy, the quality and authenticity of meat products can be evaluated through a non-destructive *in situ* method. To avoid the melting or modification of fat crystals, care should be taken regarding the density of Raman-excitation laser flux.

Instruments for Raman spectroscopy have become more accessible thanks to innovative downsizing and less expensive devices. Raman spectroscopy is being established as one of the routine methods of analyzing meat quality. In

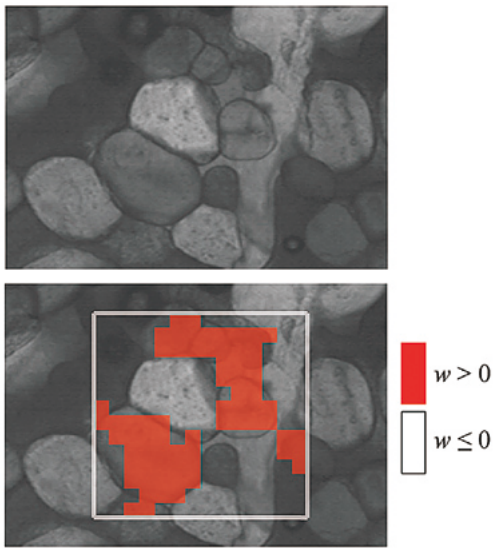

Fig. 7. Species-specific detection of pork adipocyte in beef-pork minced meat. Optical microscopic images (upper) and the area discriminated as pork (lower).
Reproduced from Motoyama et al. (2015).

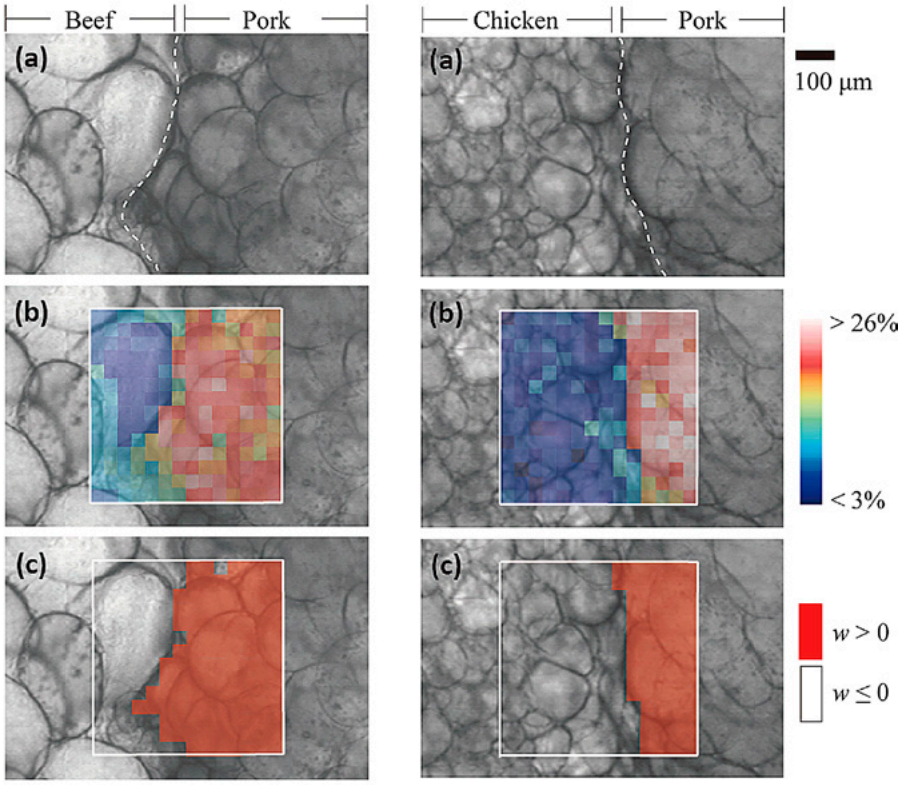

Fig. 6. Non-destructive detection of pork fat
(a) Optical microscopic images of the samples. Dashed lines indicate the border of two sample species. (b) The Raman spectrometric index corresponds to the concentration of β′-crystal polymorph relative to the total fat crystal. (c) If the Raman spectrometric index is greater than the threshold (discriminant $w > 0$), it is classified as porcine fat. Reproduced from Motoyama et al. (2015).

addition to fat crystal analysis, Raman spectroscopy has attracted increasing interest, with more research reports being submitted during the last decade in the field of meat science. Raman spectroscopy can improve our understanding of various meat properties, and consequently may improve both the quality of processing and edibility.

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