DEVELOPMENT OF RAMAN SPECTROMETRIC ON-SITE METHOD TO MEASURE SOLID FAT CONTENT

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Abstract – Crystallinity is one of the important characteristics of fats. Raman spectrometric on-site method to evaluate crystalline states of fats in meat adipose tissue has been developed; however, validation study is needed in terms of its comparability with the conventional methods which are widely used to measure fat crystallinity. With a view to establish a reliable on-site method for quantitative analysis of fat crystallinity, relation between the value measured by the Raman spectrometric method (α_C) and the one measured by a conventional NMR method (NMR-SFC) of 18 fat samples at 8 measuring temperature was investigated. It is shown that α_C and NMR-SFC were highly correlated (R^2=0.987), and by using a quadratic equation, α_C can accurately predict NMR-SFC values. To establish a Raman spectrometric method which is comparable to NMR method, validation experiments with a large number of fat samples should be further conducted.

I. 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 tissues has not been fully recognized. It is partly because of a lack of on-site technique that tells us of the crystalline states of fat.

In order to clarify the fat crystalline state in meat, a Raman spectrometric technique has been developed.[1] This technique can measure fat crystallinity on-site by using a portable spectrometer and the Raman spectrometric index for fat crystallinity is called α_C. This index is based on the intensity differences between Raman bands derived from aliphatic CH_2 moiety existing in crystal structure or in melt structure. This index; however, needs validation in an appropriate manner in terms of their comparability with the conventional methods.

Because of the importance, crystallinity is one of the essential measurement items of fat study and there are some experimental methods. Among them, nuclear magnetic resonance (NMR) techniques [2] are widely used at laboratories due to its simplicity in practical procedure. This method determines what percentage of all hydrogen nuclei in the sample, composed of hydrogen nuclei in both liquid and solid phases, is due to hydrogen nuclei in the solid phase. This percentage is called NMR-solid fat content (NMR-SFC).

We therefore conduct a preliminary study to evaluate comparability of the Raman spectrometric method with the NMR method, with the hope of establishing a reliable on-site method for fat crystallinity measurement with quantitatively comparable with data obtained at experimental laboratories.

II. MATERIALS AND METHODS

Samples

Edible fats including animal origin ones, such as beef tallow and lard, were obtained from retail markets. Detailed information of the samples is shown in Table 1. In addition to the fats, two triacylglycerol standard samples were prepared: tripalmitin (PPP, >99% purity, Sigma-Aldrich) and triolein (OOO, ~99% purity, Sigma-Aldrich). The former standard sample is totally in crystalline phase in the temperature range of the present study, and the latter one is totally in melt phase.

Solid Fat Content (NMR-SFC) measurement

SFC was measured in accordance with an official method.[2] Since samples especially beef tallow and cocoa butter contain appreciable quantities of 2-oleo-disaturated glycerides, 'METHOD II' which is suitable for the fats

Table 1 Detailed information of samples

	No.	Manu-facturer	Product character
Beef tallow	1	A	Edible beef tallow
	2	B	Edible purified beef tallow
	3	C	Fett
	4	D	Beef tallow
Lard	1	A	Purified lard
	2	B	Better lard
	3	C	Purified lard
	4	C	Pure lard
Shortening	1	E	Fluid shortening
	2	E	Shortening with cotton seed oil
	3	F	Shortening from vegetable oil
Cocoa butter	1	G	
	2	H	From Ghana
Butter	1	G	Without salt
	2	I	Without salt
	3	J	With salt

those need stabilization for reproducible results was used. Samples were prepared in NMR glass tube and stored for 15 min at 100°C, 10 min at 60°C, then 90 min at 0°C, followed by 40 h at 26°C, 90 min at 0°C, and finally 60 min at each measuring temperature. Measuring temperature were from 20°C and every 5°C up to 55°C. Metal blocks with holes for the sample glass tubes immersed in water bath were used to maintain the samples at the measuring temperatures. Bruker mq20 (Bruker BioSpin, Japan) and its application software were used to obtain NMR-SFC value. The system was calibrated by using a certified set of SFC standards (Bruker BioSpin, Germany) at the beginning of each experimental period.

Raman spectroscopic measurement

Raman spectra of the samples were obtained just before NMR-SFC measurement. A portable Raman spectrometer with 785-nm laser excitation (ProRaman-L-785C, Enwave Optronics, USA) equipped with an optic-fiber probe was used. The optic-fiber probe was introduced to the metal block where samples were maintained at the measuring temperatures. Incident angle of laser was 90° to the long axis of sample tube and backscatterd Raman light was collected by the same probe. Excitation laser power was 200 mW and signals were accumulated for 90 sec (15 sec for each 6 measurements).

Raman spectral analysis

Crystallinity of sample fats was expressed by a Raman spectrometric index, α_C, reported previously.[1]

$$\alpha_C = \frac{I_{1297}}{I_{1297}+I_{1305}} \times 100 \quad (1)$$

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 Voigt shape were assumed.

Statistical Analysis

To evaluate comparability of the Raman spectrometric index α_C with NMR-SFC, regression analysis was conducted at 5% significance by using REG procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA).

III. RESULTS AND DISCUSSION

Raman spectra of several samples at 20°C are shown in Figure 1. Very broad and intense band centered at around 1370 cm^{-1} was observed; this band originates from the glass of used sample tubes. Also, a cocoa butter sample with relatively strong yellow color exhibited baseline rise due to fluorescence from unknown origin, probably carotenes and tocopherols (Figure 1).

These broad band and baseline rise were adequately subtracted from observed spectra. Curve fitting were then applied to the spectral region of CH_2 twisting mode (Figure 2). It is shown that all spectra of the samples have spectral components at 1297- and 1305-cm^{-1}. The band at 1297 cm^{-1} originates from CH_2 moieties existing in crystalline structure and the band at 1305 cm^{-1} is from those existing in

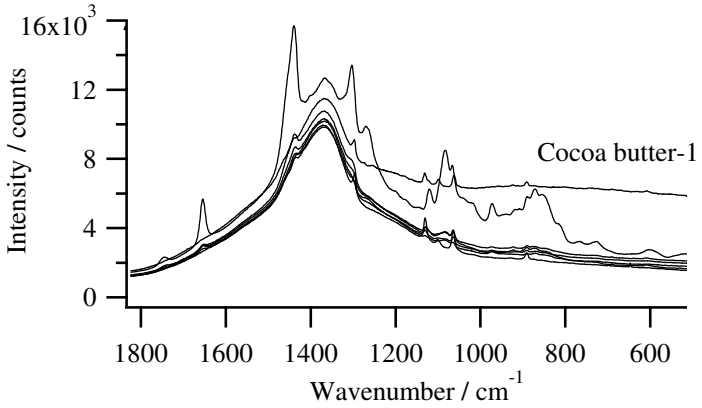

Figure 1 As observed Raman spectra of samples at 20°C.

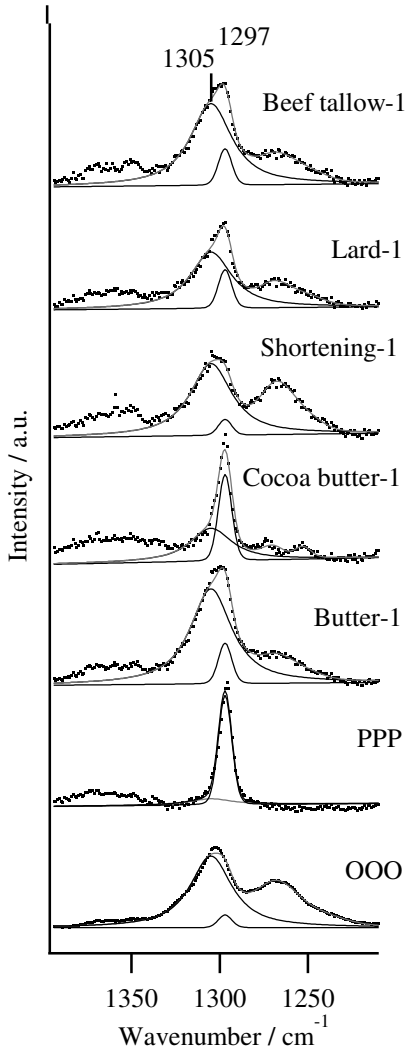

Figure 2 Curve fitting results of several samples at 20°C showing component bands at 1297- and 1305-cm^{-1}. Dotted line, spectral data; gray solid line, fitting results; black solid lines, fitting component bands at 1297 and 1305 cm^{-1}.

melt structure. From the intensities of these bands, α_C was calculated by the equation (1) and plotted against the NMR-SFC value measured by the official method (Figure 3).

From Figure 3, it can be said that the Raman spectroscopic index α_C strongly relates to NMR-SFC; however, the relation is not linear. It may be due to the difference in Raman scattering cross-section of used bands and also due to the difference in crystal types which can affect the cross section.

The relation between α_C and NMR-SFC is rather polynomial, a regression analysis using quadratic model was therefore conducted. The model was statistically significant ($P < 0.001$), and the determination coefficient, adjusted-R^2, of the regression equation was 0.987 (Figure 3).

From above results, it can be said that the Raman spectrometric index α_C is not directly equal to NMR-SFC; however, it seems to be a good predictor with the use of an adequate prediction equation. Figure 4 shows the predicted NMR-SFC values from α_C and their 95% confidence limits. α_C can predict NMR-SFC with roughly ±5% accuracy. With considering the repeatability (reliability) of the NMR method (1.3%),[2] ±5% may be a reasonably high accuracy.

IV. CONCLUSION

Relation between the Raman spectrometric index for fat crystallinity, α_C, and the NMR-SFC was investigated. They were significantly correlated and it is shown that the relation was quadratic although the reason is not clear yet. Nevertheless, α_C can predict NMR-SFC accurately from a practical point of view. To establish a Raman spectrometric method which

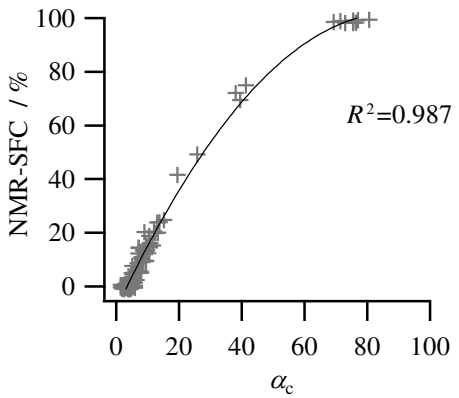

Figure 3 Relation between α_C and NMR-SFC values of the samples

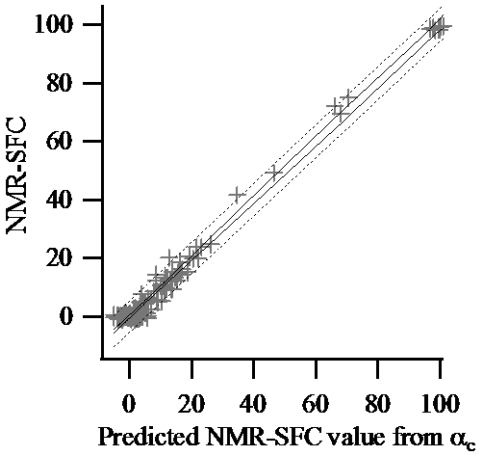

Figure 4 Relation between the predicted NMR-SFC values from α_C and the NMR-SFC. Solid line, 95% confidence limits of the regression equation; dashed line, 95% confidence limits of prediction.

is comparable to the NMR-SFC, validation experiments with a large number of fat samples should be further conducted. Also, in order to guaranty the integrity of the developing methods, creating standard samples for Raman system calibration should also be considered.

ACKNOWLEDGEMENTS

M. M. thanks Prof. Satoru Ueno at Hiroshima University for a sample and Ms. Yumiko Endo at NARO for her technical assistance during experiments. This study was supported in part by Grant-in-Aid for Scientific Research (B) 24380148 and Grant-in-Aid for Young Scientists (B) 25850098 from the Ministry of Education, Culture, Sports, Science and Technology.

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