Differentiation of Animal Fats from Different Origins: Use of Polymorphic Features Detected by Raman Spectroscopy

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Food safety requires the development of reliable techniques that ensure the origin of animal fats. In the present work, we try to verify the efficacy of using the polymorphic features of fats for discriminating animal-fat origins. We use Raman spectroscopy to collect the structural information of fat crystals. It is shown that a single Raman band at 1417 cm^{-1} successfully differentiates pork fats from beef fats. This band is known to be characteristic of the β′-polymorph of fats. Pork fats show this band because they contain the β′-polymorph after rapid cooling to 0 °C. In beef–pork-fat mixtures, this band is not detected even in the presence of 50% pork fat; an addition of beef fat to pork fat is likely to produce a mixed fat with a completely different polymorphic behavior. This method seems to have the potential to detect beef products contaminated with pork-adipose tissue.

Index Headings: **Beef tallow; Lard; Triacylglycerol; Crystal; Polymorphism; β′-polymorph; Raman spectroscopy; Vibrational spectroscopy.**

INTRODUCTION

In 2007, news shook up food safety in Japan.[1] A food-processing company added pork fats to its beef products, such as beef fats and minced beef, to obtain unfair profit. This case reminded us of the importance of reliable techniques that ensure the origin of animal foods. To detect such interspecific contamination, DNA analysis has been the most convincing method.[2,3] However, there are animal fats that are difficult to examine by DNA because of high purity[4] or degradation of DNA during high-temperature treatments in the process of food production.[5] There are other methods to detect fat-composition differences; the capillary tube method detects the melting-point depression resulting from beef-fat contamination[6] and the chromatographic method detects odd-numbered fatty acids or specific triacylglycerol (TAG) ratios derived from contaminated beef fat.[7,8] These analytical methods for detecting the differences in fat composition are also combined with chemometrics.[9] Though they are useful, they require long testing times.

The need for rapid and reliable techniques that can be used for on-site assessment has inspired an increasing use of vibrational spectroscopic approaches. Vibrational spectroscopy can provide the structural information of fats in a short time without any pretreatment of the samples. There are a few reports that compare Raman, infrared (IR), and near-infrared (NIR) spectroscopies as discrimination methods of edible fats.[10–12] Yang et al.[12] reported that Raman and IR spectroscopies were found to be more efficient than NIR for discrimination. This is reasonable because Raman and IR spectroscopies provide vibrational spectra that are often called "molecular fingerprints". IR spectroscopy has high sensitivity to water and carbon dioxide. Therefore, the IR method can be easily disturbed by a trace amount of water within the sample and/or the ambient atmosphere. On the contrary, Raman spectroscopy is not overly sensitive to water. This seems to be the primary reason that Raman spectroscopy gives the best results in predicting the concentration of added adulterants in oils.[11] Water is the most likely contaminant of edible fats; thus, Raman can be the most reliable and stable method for analyzing these fats. Most recently, Abbas et al.[13] have classified animal-fat origins using Raman spectroscopy by detecting the differences in the degree of unsaturation combined with multivariate analyses.

Natural fats are generally made up of triacylglycerols.[14,15] TAGs are the esters of a glycerol and three fatty acids (Fig. 1). These three fatty acids are stereochemically numbered ("*sn*") according to the glycerol carbon atoms to which they are attached. It is widely known that pork fats have much higher levels of palmitic acid in the *sn*-2 position than do beef fats. This difference is likely due to the substrate specificity of the enzymes involved in the TAG biosynthesis.[16] In fact, genetically determined TAG profiles contain information about the animal from which the fat originates.

One of the important features of TAGs is polymorphism. Polymorphism is defined as the existence of several crystalline forms with the same chemical composition but with different structures. In the case of TAGs, three polymorphs, α, β′, and β, are known.[17–19] These polymorphs are characterized as follows. The α form is a polymorph showing the lowest melting point, and its subcell structure is hexagonal with no ordered arrangement of the chain planes. The β′ form is a polymorph showing an intermediate melting point, and its subcell structure is orthorhombic with every second chain plane perpendicular to the planes of the rest ($O_{\perp}$). The β form is a polymorph showing the highest melting point, and its subcell structure is triclinic with all chain planes parallel. These polymorphs differ in their hydrocarbon subcell structures from one another. TAG composition and cooling-down procedures influence the crystallization of fats: how they crystallize from the melt and which polymorphs they form.

Beef fats and pork fats also exhibit polymorphism. If there are polymorphic differences between beef and pork fats, they must be reflected in their Raman spectra. In the present study, we try to verify the efficacy of using polymorphic features of fats for discriminating their origins by using Raman spectroscopy, which is highly sensitive to crystal-subcell structures.

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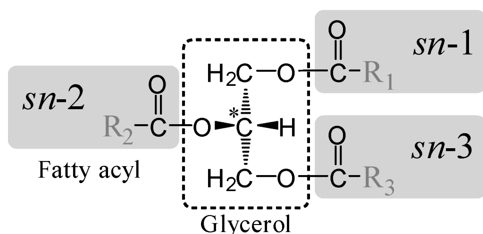


FIG. 1. Structure of triacylglycerol (TAG) and stereochemical numbering, *sn*-1, -2, and -3.

The polymorphic features of beef–pork-fat mixtures are also studied.

MATERIALS AND METHODS

Samples and TAG Profile Analysis. Seven beef fats (Beef tallows A–G, Table I) and nine pork fats (Pork fats A–I, Table I) were used. All fats were unfractionated and commercially available. They were used without further purification.

The TAG profiles of the 16 sample fats were analyzed using gas chromatography. Samples were thoroughly melted at 50 °C and 100 mg of the sample was weighed and added to 2000 μL of *n*-heptane to make 50 mg/mL sample solution. Then, 0.1 μL of solution was injected into a gas chromatograph (Shimadzu GC-17A, Kyoto, Japan) with an auto-injector (Shimadzu AOC-17). Split injection mode was selected and the ratio was 1:10. Helium was used as the carrier gas with a linear gas rate of 30 cm/s. The injector and detector temperatures were 320 and 370 °C, respectively; the oven temperature was raised from 250 to 365 °C at a rate of 5 °C/min and held at 365 °C for 5 min. The gas-chromatography capillary column was an Rtx-65TG (15 m length, 0.32 mm inner diameter, and 0.1 μm film thickness) (Restek, Bellefonte, PA). Signals were detected with a flame-ionization detector. The reference material IRMM-801 (IRMM, Geel, Belgium) was used for peak identification and determination of the calibration factor of each triacylglycerol. The chromatographic peaks, detected after an injection time of 14 min, which corresponded to the TAGs with acyls'-carbon-atoms number > 40, were integrated to calculate the total amount of TAG. Each TAG quantity was expressed as the ratio to the total. All samples were analyzed in duplicate.

Raman Spectroscopic Measurement and Analysis. The samples were thoroughly melted at 50 °C and 5 μL of melt was put on a CaF_2 glass slide (0.3 mm thickness). The slide was set in a cryostat (Linkam 10021, Tadworth, Surrey, UK) and nitrogen atmosphere was provided in order to prevent autoxidation. First, the sample was heated at 80 °C for 1 min to erase any crystal memory. Then crystals were prepared by cooling down to incubation temperatures (10, 0, −10, and −20 °C) at a rate of −20 °C/min and held for 5 min. Raman spectra were measured after the incubation, and the samples were kept at the incubation temperature in a cryostat during the measurements.

Raman scattering was excited with the 785-nm line of a Ti-sapphire laser (Spectra Physics 3900S, Newport, Santa Clara, CA). The back-scattered Raman light from the sample was collected using an objective lens (LUCPlanFLN20x, Olympus, Tokyo, Japan) and measured with a spectrometer (Chromex 250i, Bruker Optik GmbH, Ettlingen, Germany) and a charge-coupled device (CCD) detector (400×1340 pixels, Spec-10 400BR(LM), Roper, Sarasota, FL). The laser power was measured using a power meter with a photodiode sensor (PD300, Ophir Optronics, Jerusalem, Israel). It was 30 mW at the sample point. Three measurements with exposure times of 60 s were accumulated. Spectral resolution was 3.8 cm^{-1}. The laser focal point was about 11 μm in diameter with 60-μm spatial resolution in the horizontal direction. Measurements were made in duplicate. The average spectra are shown in the following. The spectra were baseline subtracted and deconvoluted with the slit function of the spectrometer (a Gaussian function, the half width at half-maximum was 1.9 cm^{-1}) with the use of a triangular apodizing function. The deconvoluted spectra were normalized with the CH_2-scissors bands (1410–1480 cm^{-1}) in order to eliminate the effect of laser power fluctuation. The intensity of the 1417 cm^{-1} band was acquired by band fitting using a Lorentzian function and the data were assessed with Welch's analysis of variance and the *t*-test.

TABLE I. Samples and their detailed information.

Sample name		Product name	Identity
Beef fat	A	"Beef tallow"	Sigma-Aldrich, 03-0660
	B	"Edible beef tallow"	Manufacturer 1, product A, lot. A
	C	"Edible beef tallow"	Manufacturer 1, product A, lot. B
	D	"Refined beef tallow"	Manufacturer 2, product A
	E	"Edible refined beef tallow", JAS[a]	Manufacturer 3, product A, lot. A
	F	"Edible refined beef tallow", JAS	Manufacturer 3, product A, lot. B
	G	"Hett"	Manufacturer 4, product A
Pork fat	A	"Pork fat"	ERM[b]-BB444
	B	"Pork fat"	ERM-BB446
	C	"Pork fat"	BCR[c]-430
	D	"Refined lard", JAS	Manufacturer 1, product B, lot. A
	E	"Refined lard", JAS	Manufacturer 1, product B, lot. B
	F	"Refined better lard", JAS	Manufacturer 3, product B, lot. A
	G	"Refined better lard", JAS	Manufacturer 3, product B, lot. B
	H	"Refined lard"	Manufacturer 4, product D, lot. A
	I	"Refined lard"	Manufacturer 4, product D, lot. B

[a] JAS: Japanese Agricultural Standard.
[b] ERM: European Reference Material.
[c] BCR: Community Bureau of Reference.

TABLE II. TAG profiles of the samples. Units: g/100-g total TAG.

		TAG molecule[a]						
		PPP	MOP	PPS	POP	PLP	PSS	POS
Beef tallow	A	2.0 ± 0.0[b]	4.2 ± 0.0	2.5 ± 0.0	9.4 ± 0.1	0.9 ± 0.1	1.6 ± 0.0	12.3 ± 0.0
	B	3.6 ± 0.0	4.1 ± 0.0	2.4 ± 0.0	10.7 ± 0.0	1.1 ± 0.2	1.4 ± 0.0	10.1 ± 0.1
	C	3.8 ± 0.0	4.4 ± 0.0	2.6 ± 0.0	11.6 ± 0.0	1.3 ± 0.2	1.5 ± 0.0	10.6 ± 0.0
	D	6.1 ± 0.0	4.4 ± 0.0	2.8 ± 0.1	12.4 ± 0.2	1.2 ± 0.2	1.3 ± 0.0	10.2 ± 0.1
	E	2.5 ± 0.0	4.5 ± 0.0	2.9 ± 0.0	9.8 ± 0.0	1.1 ± 0.1	2.2 ± 0.0	12.1 ± 0.0
	F	2.1 ± 0.0	4.8 ± 0.0	2.6 ± 0.1	9.4 ± 0.1	1.1 ± 0.0	1.9 ± 0.0	11.3 ± 0.2
	G	1.6 ± 0.0	3.7 ± 0.1	2.3 ± 0.0	8.8 ± 0.0	1.0 ± 0.2	1.7 ± 0.0	13.1 ± 0.2
	Median	2.5	4.4	2.6	9.8	1.1	1.6	11.3
Pork fat	A	0.7 ± 0.0	1.7 ± 0.1	2.1 ± 0.1	9.0 ± 0.1	1.2 ± 0.1	1.9 ± 0.0	20.3 ± 0.0
	B	0.7 ± 0.0	1.8 ± 0.0	2.1 ± 0.0	9.2 ± 0.0	1.2 ± 0.2	1.9 ± 0.0	20.2 ± 0.0
	C	0.5 ± 0.0	1.7 ± 0.1	1.6 ± 0.1	7.5 ± 0.1	1.8 ± 0.1	1.5 ± 0.0	18.6 ± 0.0
	D	1.0 ± 0.0	2.2 ± 0.0	2.4 ± 0.0	8.9 ± 0.0	2.0 ± 0.2	2.2 ± 0.0	19.8 ± 0.1
	E	1.0 ± 0.0	2.2 ± 0.0	2.4 ± 0.0	8.9 ± 0.1	1.8 ± 0.1	2.2 ± 0.0	19.5 ± 0.0
	F	0.9 ± 0.0	2.1 ± 0.1	2.4 ± 0.0	9.1 ± 0.1	1.6 ± 0.1	2.1 ± 0.0	20.1 ± 0.1
	G	0.9 ± 0.0	2.2 ± 0.0	2.4 ± 0.0	9.1 ± 0.1	2.0 ± 0.2	2.1 ± 0.0	19.6 ± 0.1
	H	0.7 ± 0.0	1.9 ± 0.0	1.9 ± 0.0	8.4 ± 0.0	1.0 ± 0.2	1.6 ± 0.0	18.9 ± 0.1
	I	0.8 ± 0.0	1.8 ± 0.0	2.3 ± 0.0	8.5 ± 0.0	1.3 ± 0.0	2.3 ± 0.0	19.9 ± 0.1
	Median	0.8	1.9	2.3	8.9	1.6	2.1	19.8

[a] TAGs shown are the identifiable major species present.
[b] Values represent the mean value of two replicates with standard deviation.

RESULTS AND DISCUSSION

Fatty acyls are abbreviated as follows: (M) myristic acyl; (P) palmitic acyl; (O) oleic acyl; (S) stearic acyl; (L) linoleic acyl; and (A) arachidic acyl. TAG molecular species are expressed with three-letter notation using the abbreviated letters, e.g., POS. "POS" can include six TAG species: *sn*-POS, *sn*-PSO, *sn*-OPS, *sn*-OSP, *sn*-SPO, and *sn*-SOP, while "*sn*-POS" means the specific TAG species: *sn*-1-palmitoyl-2-oleoyl-3-stearoyl-glycerol.

TAG Profile of the Samples. The TAG profiles of tested samples are presented in Table II. Though variances among previous studies exist, the overall tendency of the profiles of the present study is in agreement with these reports.[8,20] Referring to these studies, *sn*-OPO is the most abundant TAG species in the present sample set of pork fats. Its concentration is estimated to be approximately 22% (w/w) of the total TAG; *sn*-OPO accounts for more than 95% of POO in pork fats,[20] and the POO concentration of the present study is about 23%. This POO concentration (23%) has been derived by using its relative amount (77%)[8] to POO+PLS (30.1% of the total TAG, Table II). On the other hand, *sn*-POO/OOP is the major component in the beef fats. Its concentration is estimated to be approximately 22% (w/w) of the total TAG; *sn*-POO/OOP accounts for ~86% of POO,[20] which corresponds to 25.1% of POO+PLS in beef fats.[8] The second major TAG in the beef fats is *sn*-POS/SOP, whose concentration is estimated to be ~7% (w/w) of the total TAG; *sn*-POS/SOP accounts for 61%[20] of POS (11.3% of the total TAG, Table II).

Raman Spectra of Fat Crystals. During the process of cooling, melts of beef and pork fats begin to crystallize when the temperature reaches approximately 20 °C. Both fats show granular morphologies composed of a large number of small crystals. It is difficult to identify polymorphic forms only by microscopic images because a polymorphic form could appear as different crystal sizes and different crystal shapes.[21]

The Raman spectra of beef fat A and pork fat A at different incubation temperatures are compared in Fig. 2a. Though these Raman spectra resemble one another, the pork fat shows a shoulder at 1417 cm^{-1} (Fig. 2b), while the beef fat does not exhibit this band at the incubation temperature of ≥0 °C. This band is assigned to the CH_2-scissors mode characteristic of the $O_\perp$-subcell structure.[22] In terms of TAG, it is the β′-polymorph that has the $O_\perp$ subcell structure to give this band.[23] It is therefore shown that the pork fat contains the β′-polymorph under the present experimental conditions. It is widely known that pork fats tend to be crystallized in β-form.[17,24] Due to the highly biased distribution of palmitic acyl at the *sn*-2 position in pork fats, they are easy to pack and reorder to the most orderly and stable polymorphic form, β. The metastable β′ polymorph formation in the present study is most likely to be caused by the rapid cooling rate and short incubation time. Campos et al.[25] also reported that rapid cooling induced β′ in a pork fat. Nucleation and growth of the metastable form normally predominate in fat crystallization, and reformation to the most stable polymorph is a kinetic process that takes time. The reformation seems not to be completed within the 5 minutes of incubation used in the present study.

In the beef fat, cooling to −20 °C produces the β′-polymorph (Fig. 2b). This observation is in accordance with the previous study,[26] which reported that the rapid cooling to −25 °C produces the β′-polymorph in beef fat. On the contrary, the incubation temperatures of 10, 0, and −10 °C induce a small amount of β′ even though the melting point of β′ in beef fats is higher than these temperatures.[26] It might be because the cooling to above −20 °C provided insufficient super-cooling for the beef fat to crystallize in the β′ form. For TAG crystallization, it is known that melts should be cooled well below the melting point because of the free energy penalty associated with crystal formation.[27] More stable polymorphs have higher free energy penalty and therefore they need more super-cooling to crystallize. The incubation temperatures above −20 °C are likely to form less stable α-polymorphs of the beef fat.

The other differences between the spectra of the beef fat and those of the pork fat are not sensitive to the polymorphic differences. Relatively large differences are observed in the C–C stretch region (1140–1040 cm^{-1}) and the C=O stretch

TABLE II. Extended

TAG molecule[a]								
POO (OPO) + PLS	PLO	SSS	SOS	SOO	OOO+SLS	SLO	SOA	AOO
25.9 ± 0.1	4.0 ± 0.1	1.0 ± 0.0	3.8 ± 0.1	8.3 ± 0.1	4.9 ± 0.3	1.0 ± 0.2	0.1 ± 0.0	···
23.3 ± 0.1	4.4 ± 0.1	0.8 ± 0.0	2.6 ± 0.1	6.0 ± 0.1	4.4 ± 0.2	0.9 ± 0.2	6.2 ± 0.1	0.1 ± 0.0
25.1 ± 0.0	4.7 ± 0.0	0.9 ± 0.0	2.8 ± 0.0	6.5 ± 0.0	4.5 ± 0.2	1.0 ± 0.1	0.1 ± 0.1	···
23.4 ± 0.1	4.5 ± 0.1	0.8 ± 0.0	2.6 ± 0.0	5.9 ± 0.0	4.7 ± 0.1	0.9 ± 0.1	···	···
25.2 ± 0.1	4.7 ± 0.1	1.2 ± 0.0	2.9 ± 0.0	6.6 ± 0.1	4.3 ± 0.0	1.2 ± 0.1	···	···
24.9 ± 0.4	4.4 ± 0.1	1.1 ± 0.0	3.2 ± 0.1	7.2 ± 0.1	4.5 ± 0.1	1.2 ± 0.0	···	···
26.8 ± 0.2	4.2 ± 0.0	1.0 ± 0.0	4.0 ± 0.1	8.8 ± 0.0	5.1 ± 0.0	1.0 ± 0.1	0.1 ± 0.0	···
25.1	4.4	1.0	2.9	6.6	4.7	1.0	0.1	···
30.5 ± 0.0	8.8 ± 0.0	0.5 ± 0.0	1.2 ± 0.0	3.7 ± 0.1	3.3 ± 0.0	1.9 ± 0.0	···	···
30.6 ± 0.1	8.9 ± 0.0	0.5 ± 0.0	1.2 ± 0.0	3.5 ± 0.0	3.3 ± 0.0	2.0 ± 0.1	···	···
30.1 ± 0.1	11.1 ± 0.1	0.5 ± 0.0	1.1 ± 0.0	3.5 ± 0.0	3.2 ± 0.0	2.2 ± 0.0	0.1 ± 0.0	···
29.0 ± 0.0	8.5 ± 0.1	0.5 ± 0.0	1.5 ± 0.1	4.0 ± 0.1	3.3 ± 0.0	1.9 ± 0.1	···	···
28.7 ± 0.2	8.3 ± 0.1	0.5 ± 0.0	1.6 ± 0.0	4.1 ± 0.0	3.6 ± 0.1	1.9 ± 0.0	···	···
29.3 ± 0.1	8.5 ± 0.1	0.4 ± 0.1	1.4 ± 0.0	3.9 ± 0.1	3.4 ± 0.2	1.9 ± 0.0	···	···
29.2 ± 0.1	8.3 ± 0.1	0.5 ± 0.0	1.4 ± 0.0	3.7 ± 0.1	3.5 ± 0.0	1.9 ± 0.2	···	0.1 ± 0.0
30.9 ± 0.1	8.8 ± 0.1	0.5 ± 0.1	1.5 ± 0.0	4.4 ± 0.0	3.9 ± 0.1	2.2 ± 0.0	···	···
30.1 ± 0.1	8.9 ± 0.1	0.5 ± 0.0	1.5 ± 0.0	4.0 ± 0.0	3.5 ± 0.0	2.2 ± 0.1	···	···
30.1	8.8	0.5	1.4	3.9	3.4	1.9	···	···

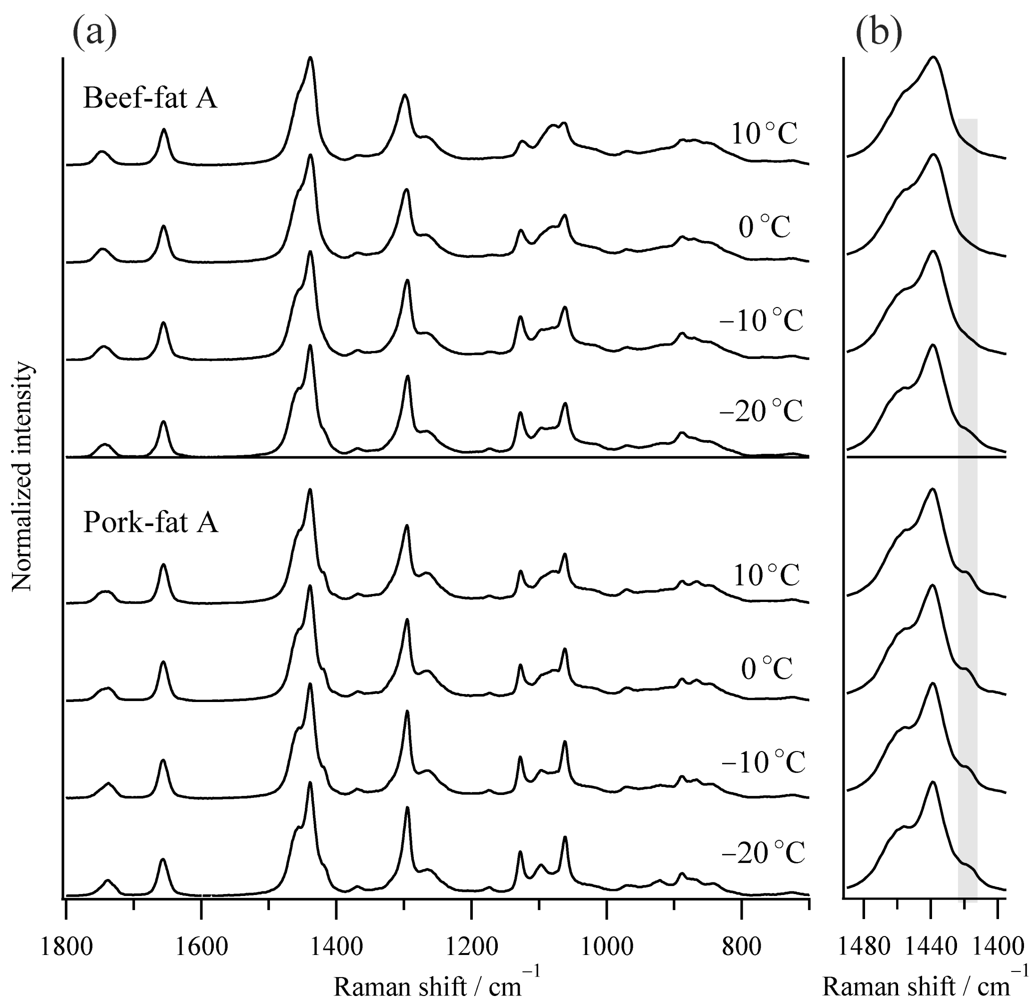


Fig. 2. Raman spectra of beef and pork fats at each incubation temperature. (**a**) Spectra of beef fat A and pork fat A. These two fats have the medium TAG composition within each animal-fat group (see Table II). (**b**) Enlarged spectra of the CH_2-scissors region corresponding to each left-hand-side spectrum. Shaded region indicates the position ~1417 cm^{-1}.

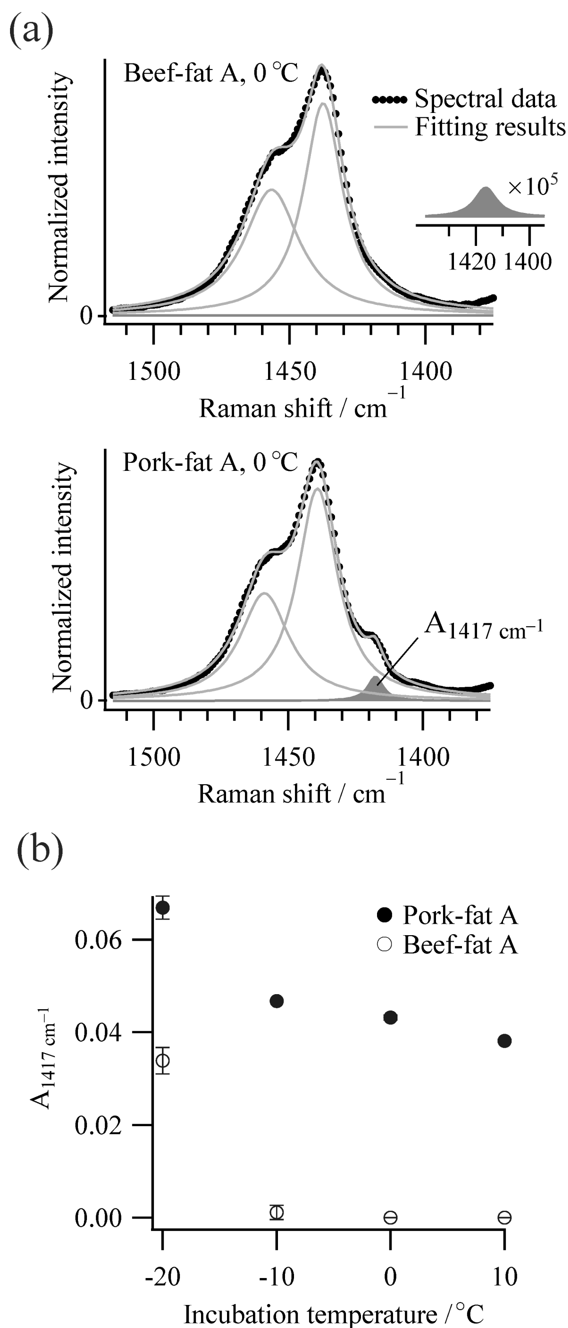


FIG. 3. The 1417 cm^{-1} band intensities ($A_{1417cm^{-1}}$) of both fats. (**a**) Intensities are acquired by Lorentzian band fitting. (**b**) Relation between $A_{1417cm^{-1}}$ and incubation temperatures.

region (1770–1720 cm^{-1}). The intensities of these conformation-sensitive bands have been employed as a measure of conformational order of TAG.[28,29] However, the significant amount of liquid TAG (i.e., TAG in random form) within the sample masks the band features due to the crystal polymorphs. At the temperature range of the present experiment, beef fats and pork fats are in the form of crystalline suspensions in liquid-form TAG.

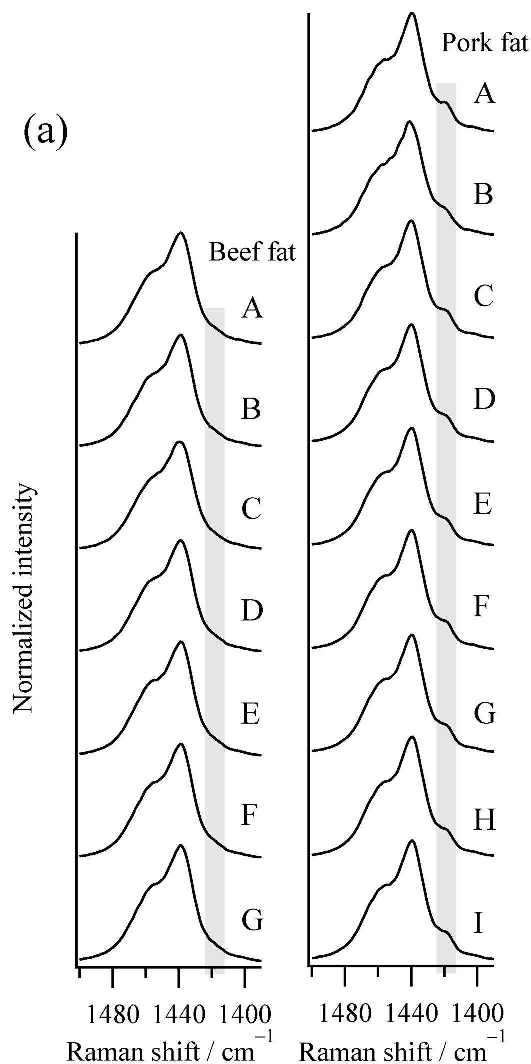
The 1417 cm^{-1} band intensities ($A_{1417cm^{-1}}$) of both fats are acquired by band fitting (Fig. 3a) and their dependence on incubation temperatures is shown (Fig. 3b). The difference in $A_{1417cm^{-1}}$ between the pork and beef fats is most remarkable when the incubation temperature is −10∼0 °C; therefore, this temperature range seems to be optimal for discriminating these two fats. The following experiments have been conducted using 0 °C as the incubation temperature.

Figure 4a shows the Raman spectra of the seven beef fats and the nine pork fats measured at the incubation temperature of 0 °C. The 1417 cm^{-1} band is easily detected in all pork fats, while it is very weak in beef fats. The $A_{1417cm^{-1}}$ value of each sample is acquired using band fitting and plotted for each fat group in Fig. 4b. The variances of the $A_{1417cm^{-1}}$ values of these two groups are unequal; therefore, Welch's *t*-test is conducted to find whether the averages are different. The average $A_{1417cm^{-1}}$ value of pork fats is statistically higher than that of beef fats at a significance level of $P < 0.0001$ (Fig. 4b). It is therefore shown that this band successfully discriminates the origins of the present samples. The difference in polymorphic features enables Raman spectroscopy to distinguish these two fats using a single band.

Then, we evaluated the sensitivity of this band to detect pork-fat contamination in a beef fat. Beef fat A and pork fat A, which have moderate TAG compositions within each fat group (Table II), were thoroughly melted and mixed using a vortex mixer to prepare beef–pork mixed fats with different pork-fat concentrations. The 1417 cm^{-1} band intensities measured for 15 different mixing ratios are plotted in Fig. 5. When pork fat concentrations are below 50%, the band intensities are very small and difficult to detect. It is indicated that the β′-polymorph scarcely exists even in the presence of 50% pork fat. Furthermore, the approximated straight line of the band intensity ratio does not cross the point of origin (dashed line in Fig. 5). Considering the fact that the pork fat contains a large amount of β′ forming TAGs, this line should cross the point of origin. It seems that the addition of the beef fat markedly disturbs the β′-polymorph formation of these TAGs. Concerning *sn*-OPO, the most abundant TAG in the pork fat, an interesting study has been carried out by Minato et al.[30] They report the thermal and structural properties of the binary mixture of *sn*-OPO and *sn*-POP. They conclude that these two TAGs form a molecular compound (OPO/POP compound) at 1:1 molar ratio and this compound behaves just like a *de novo* TAG species. The OPO/POP compound forms α- and β-polymorphs but does not form β′.[30] This OPO/POP compound formation is most likely to be the reason that the addition of the beef fat disturbs β′ formation in the pork fat: the pork-fat TAGs that can form molecular compounds (e.g., *sn*-OPO) are likely to produce compounds with the TAGs in added beef fats. Further studies are needed to elucidate the mechanism that underlies the drastic change in the phase behavior of pork fats when mixed with beef fats.

CONCLUSION

It has been shown that Raman spectroscopy can distinguish beef fats and pork fats using a single marker band at 1417 cm^{-1}. This band is derived from the β′-polymorph of fats. To discriminate pork and beef fats, the sample is thoroughly melted then rapidly cooled down (−20 °C/min in this study) to iced-water temperature (0 °C), incubated for 5 min, and then checked for the existence of the Raman band. Obviously, this method can be applied to adipose tissues.[31] Pork adipose tissue minced with beef meat will also be examined with the present method. In this case, the pork fat tends to exist within cells and avoid complete mixing with the beef fat; therefore, the



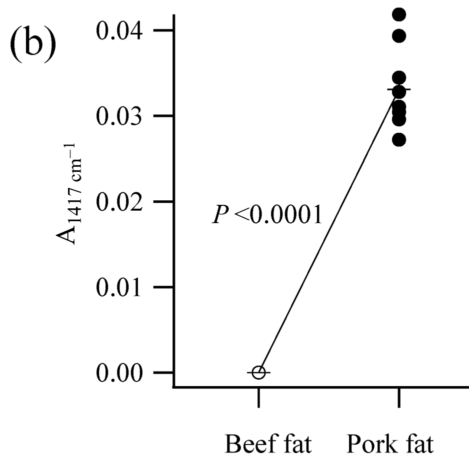


FIG. 4. (**a**) Raman spectra of the CH_2-scissors region of all samples after rapid cooling down to incubation at 0 °C. (**b**) The 1417 cm^{-1} band intensity ($A_{1417cm^{-1}}$) of each sample is plotted for each fat group. The average $A_{1417cm^{-1}}$ value of each fat group (indicated by the line) is also plotted. The pork fats have statistically higher $A_{1417cm^{-1}}$ values than the beef fats at a significance level of $P < 0.0001$.

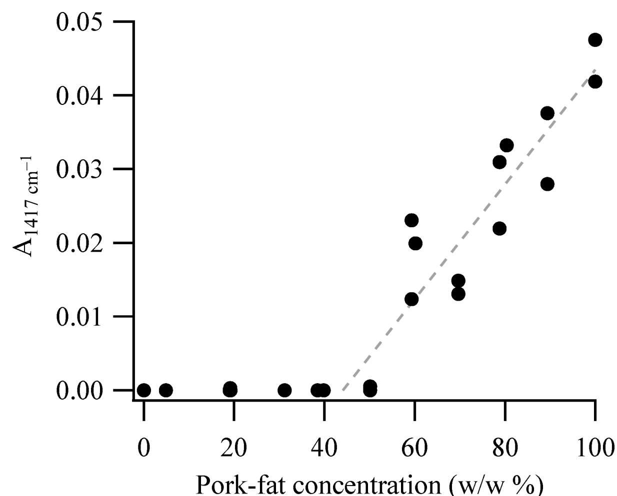


FIG. 5. Relation between $A_{1417cm^{-1}}$ and pork-fat concentration. The dashed line is the approximated straight line fitted with the data of 60–100% pork-fat concentrations.

detection sensitivity will be much higher than that for the fat mixture.

The thermal history is the key factor that makes this method feasible. If an appropriate incubation temperature is found, other fats can also be discriminated by their polymorphic features. This new idea of using polymorphic features to discriminate the fat origin will contribute to refining the existing spectroscopic methods. IR spectroscopy can also employ this idea: IR absorption bands of the CH_2-rock and CH_2-scissors modes also show distinctive bands derived from $O_{\perp}$-subcell structure of the β'-polymorph.[22]

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