RAMAN SPECTROSCOPIC DISCRIMINATION OF ANIMAL FATS ORIGINS: USE OF POLYMORPHIC PROPERTIES

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Abstract— **From the standpoint of animal-food safety, the development of reliable techniques that insure the animal-fat origins is highly important. In the present work, the efficacy of the polymorphic features of fats for discriminating animal-fat origins is verified. Raman spectroscopy was used to collect the structural information of fat polymorphs. It is shown that a single Raman band at 1417 cm^{-1} successfully discriminates pork fats from beef fats. This band is known to be characteristic to the orthorombic subcell structure of fat crystals. The β'-polymorph which was produced in the pork fats seems to be the origin of this band.**

Index Terms— **Beef tallow, lard, triacylglycerol, crystal, polymorphism, β'-polymorph, vibrational spectroscopy, rapid method.**

I. INTRODUCTION

In 2007, a news shook up food safety in Japan [6]. A food-processing company added pork fats to its beef products, such as beef fats and minced beef, for getting unfair profit. This case reminded us of the importance of reliable techniques that insure the animal-food origins. The need of rapid and reliable techniques that fit for on-site assessment has urged an increasing use of vibrational spectroscopic approaches. Vibrational spectroscopy can give us the structural information of fats in a short time without any pretreatment of the samples.

Animal fats are mainly made up of triacylglycerols (TAGs) [2, 13]. 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 well-known [3, 7, 8]. Beef fats and pork fats also exhibit polymorphism. If there are polymorphic differences between beef and pork fats, they must be reflected in their vibrational spectra.

In the present study, we try to verify the efficacy of polymorphic features of fats for discriminating their origins by using a vibrational spectroscopy, Raman spectroscopy, that is highly sensitive to crystal structures.

II. MATERIALS AND METHODS

A. Samples

Seven beef fats and nine pork fats were used. All fats were unfractionated and commercially available. They were used without further purifications.

B. Raman spectroscopic measurement

The samples were thoroughly melted at 50°C and 5-μl melt was put on a slide glass. The slide glass was set in a cryostat (Linkam 10021, Tadworth, Surrey, UK) and nitrogen atmosphere was provided in order to avoid autoxidation. Firstly, the sample was heated at 80°C for 1 min to erase any crystal memories. Then crystals were prepared by cooling down to incubation temperatures (10, 0, −10 and −20°C at a rate of −20°C/min and hold for 5 min. Raman spectra were measured after the incubation and the samples were kept at the incubation temperature during the measurements.

The 785-nm line of a Ti-sapphire laser (Spectra Physics 3900S, Newport, Santa Clara, CA, USA) was used to excite Raman scattering. The back-scattered Raman light was collected by an objective lends (LUCPlanFLN20x, Olympus, Tokyo, Japan) and measured with a spectrometer (Chromex 250i, Bruker Optik GmbH, Ettlingen, Germany) and a CCD

detector (Spec-10 400BR(LM), Roper, Sarasota, Florida, USA). The laser power at the sample was 30 mW and the accumulation time was 60 sec. Measurements were made in duplicate.

III. RESULTS AND DISCUSSION

Melts of beef- and pork-fats begin to crystallize when the temperature goes down to approximately 20°C. It is difficult to identify polymorphic forms only by microscopic images because a polymorphic form could appear in different crystal sizes and different crystal shapes [4].

The Raman spectra of a beef-fat sample and a pork-fat sample at different incubation temperatures are compared in Fig. 1. Though these Raman spectra resemble one another, the pork fat shows a distinctive band at 1417 cm^{-1}, while the beef fat exhibits this band only at the incubation temperature of 20°C. This band is assigned to the CH_2-scissors mode characteristic of the orthorhombic-subcell structure [5]. Regarding TAGs, it is the β'-polymorph that has the orthorhombic subcell structure to give this band [12]. 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 [3, 14]. Due to the highly-biased distribution of palmitic acyl at *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, Narine and Marangoni (2002) also reported 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 the kinetic process that takes time. The reformation seems not to be completed within 5-min incubation in the present study.

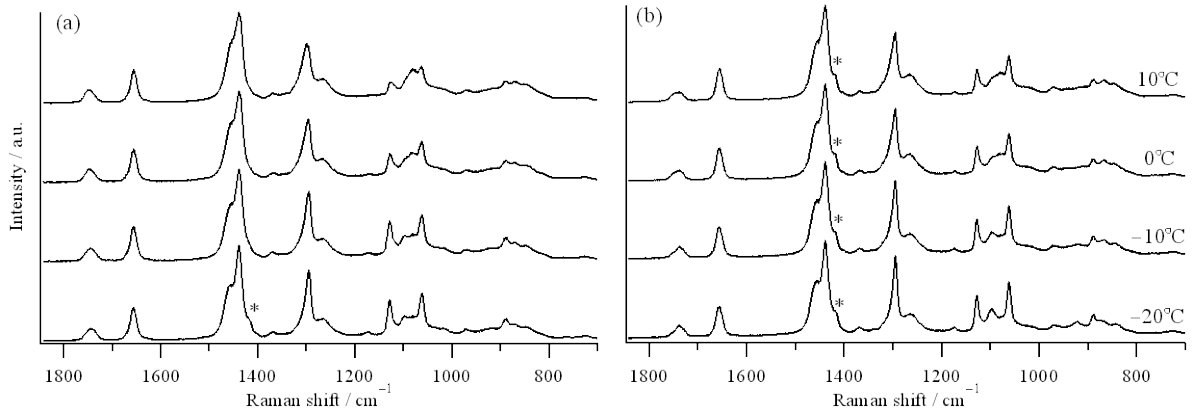

Fig. 1 Raman spectra of beef- and pork-fats at each incubation temperature. The spectra have been normalized with the CH_2-scissors bands (1480–1400 cm^{-1}) to eliminate the effect of laser power fluctuation. (a) beef fats, (b) pork fats. Asterisk (*) indicates the band at ~1417 cm^{-1}.

In the beef fat, only cooling to −20°C produced the β'-polymorph (Fig. 1). This observation is in accordance with the previous study that has reported the rapid cooling to −25°C produced the β'-polymorph in beef fat [11]. On the contrary, the incubation temperatures of 10, 0 and −10°C do not induce β' even though the melting point of β' in beef fats is higher than these temperatures [11]. It might be because the cooling to above −20°C provided insufficient supercooling 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 [9]. More stable polymorphs have higher free energy penalty and therefore they need more supercooling to crystallize. The incubation temperatures above −20°C are likely to form less stable α-polymorph 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 difference. Relatively large differences are observed in the C–C stretch- (1140–1040 cm^{-1}) and the C=O stretch-region (1770–1720 cm^{-1}). The intensities of these conformation-sensitive bands have been employed as a measure of conformational order of TAG [1, 15]. 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.

Figure 2 shows the typical Raman spectra of the beef fat and the pork fat measured at the incubation temperature of 0°C. The 1417 cm^{-1} band is detected in all pork fats but not in any of beef fats. It is shown that this band successfully discriminates the origins of the present sample sets. The difference in polymorphic features enables Raman spectroscopy to distinguish these two fats by a single band.

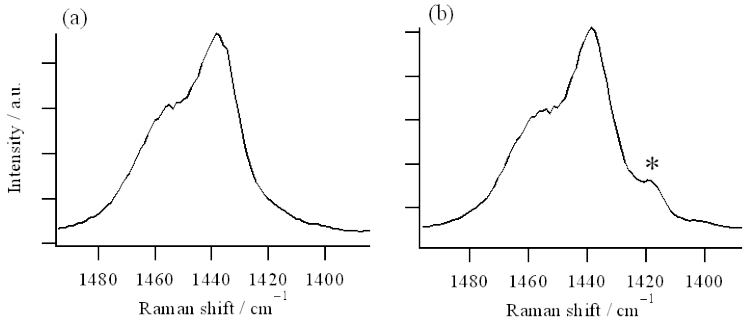

Fig. 2 Raman spectra of the CH_2-scissors region of the samples after rapid cooling down to and incubation at 0°C. The typical spectrum of beef fats (a) and pork fats (b). Asterisk (*) indicates the band at ~1417 cm^{-1}.

IV. CONCLUSION

It is shown that Raman spectroscopy is able to distinguish beef fats and pork fats by a single marker band at 1417 cm^{-1}. This band is derived from the orthorhombic subcell structure of β'-polymorph of fats. To discriminate pork and beef fats, the sample is once thoroughly melted then rapidly cooled down (–20°C/min in this study) to the iced-water temperature (0°C), incubated for 5 min, and then checked the existence of the Raman band. Obviously, this method can be applied to adipose tissues [10]. This new idea of using polymorphic features to discriminate the fat origin will contribute to refine 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 orthorhombic-subcell structure of the β'-polymorph [5].

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