論文の内容の要旨

論文題目　　　Structure and phase characterization of triacylglycerols by Raman spectroscopy
（ラマン分光法によるトリアシルグリセロールの構造および相挙動解析）

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Chapter 1: Introduction

Triacylglycerols (TAGs) are one of the main forms of energy storage in living organisms. Natural fats, which are basically multicomponent TAG systems, are the major components of food. They are also used as matrices of medicine and cosmetics. They are made up of more than 30 TAG species and their major constituent fatty acid is generally oleic acid. Industrial demands promote the studies on thermophysical properties of the multicomponent TAG systems for a long time; however, the whole picture of their phase behavior has not been completely understood yet because of the complexity.

In order to understand the phase behaviors of these complex systems, many studies have been carried out for simple TAG systems as the models. Powder X-ray diffraction has been the most used experimental approach and has revealed interesting phenomena such as crystal polymorphism and "molecular compound" formation. However, the technique suffers from serious limitations when studying coexistence of multiple phases.

To study these phenomena within multicomponent TAG systems, Raman spectroscopy has a distinct advantage. Raman spectrum as a whole is often called "molecular finger print" which is distinctive to a unique phase. By using this characteristic, one can extract the information of the phases coexisting in a complex system. Also, lipids are appropriate molecules for Raman spectroscopy because their large polarizability volumes give strong Raman scattering. Their structural changes are reflected in the spectra with high sensitivity.

In this study, multicomponent TAG systems are investigated by the use of Raman spectroscopy to characterize the phases occurring in these systems. For this purpose, the structure and phase behavior of TAGs are firstly summarized with emphasis on the recent developments (Chapter 2) and then their Raman spectral features are described (Chapter 3). Based on these, the phase behavior of some multicomponent systems are studied by Raman spectroscopy (Chapters 4 and 5).

Chapter 2: Structure and phase behavior of TAGs

TAGs possess the basic structure of lipids: a glycerol backbone and acyl chains attached to it (Fig. 1a). The chemical composition of the acyl chains of natural fats is genetically determined and affects their phase behavior.

One of the important phase behaviors of TAGs is polymorphism (Fig. 1b). Three polymorphic phases, α, β*'* and β, are generally observed. α and β*'* are the metastable phases and β is the most stable one. The polymorphic phases differ in their hydrocarbon subcell structure.

It is also known that TAGs form "molecular compound" in their binary systems (Fig. 1c). A molecular compound behaves like a new, pure TAG species with unique phase behavior that differs from those of its component TAGs. The formation of a molecular compound is thought to occur in terms of the specific interactions between oleic acyl moieties of the component TAG molecules.

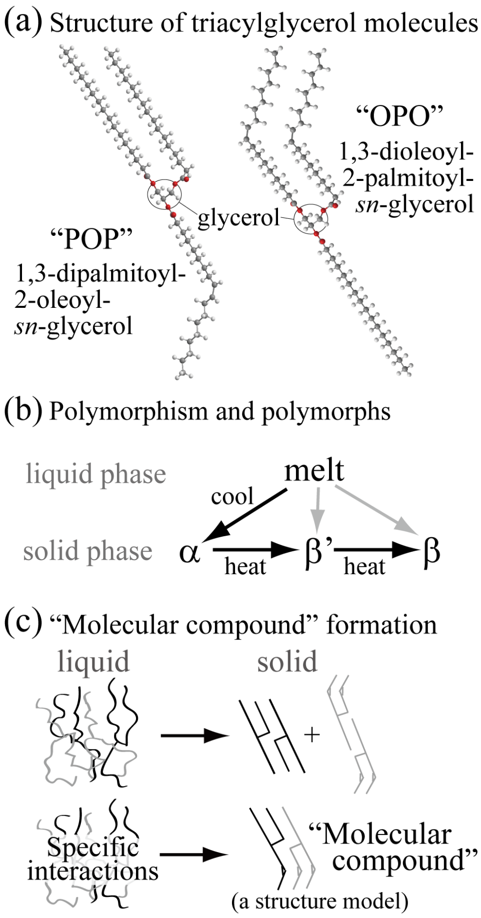

Fig. 1 Structures and interesting phase behaviors of TAGs

Chapter 3: Raman spectra of TAGs

Raman spectral features of TAGs are described in relation to TAG structure of each phase. On the basis of the spectroscopic data of basic molecules such as polyethylene, paraffin, *n*-alkanes and fatty acids, the detailed TAG structures can be characterized from Raman spectra. Their spectra are largely determined by acyl moieties that dominate the compositions. The conformation and inter-chain interaction of acyl moieties differ among the phases and are reflected in their Raman spectra. There is little information on the glycerol conformation so far; however, some spectral regions do indicate the difference in this moiety for the different polymorphic phases.

Chapter 4: Investigation of molecular compound formation in a TAG binary system

1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol (POP, Fig. 1a) and 1,3-dioleoyl-2-palmitoyl-*sn*-glycerol (OPO, Fig. 1a) binary system was investigated using Raman spectroscopy.

POP and OPO were completely melted and mixed to prepare the samples with different molar ratios of POP and OPO. The crystals of the samples were prepared by cooling down the melt to 4°C followed by incubation. Raman spectra of the polycrystal were measured by a 532-nm excitation Raman spectrometer which is developed in the laboratory. The Raman spectral data of the samples are assembled into a matrix and subjected to singular value decomposition to analyze the number of independent spectral components. The spectrum and the concentration profile of each component were reconstructed under the constraints in order to minimize ambiguities.

It is found that two spectral components are not enough to explain the data set. On the other hand, three components successfully explain the data. Their concentration profiles and spectra are shown in Fig. 2. From these results, the existence of the third component in the binary system is shown spectrometrically. The components 1 and 2 are POP and OPO, respectively. The third component, component 3, is thought to be the POP-OPO molecular compound. However, it seems that the compound is formed at the molecular ratio of POP:OPO=1:2 and not 1:1 as reported previously.[1] This is may be due to the difference in thermal treatment on crystal preparation. Further studies are needed to identify this component and its structure

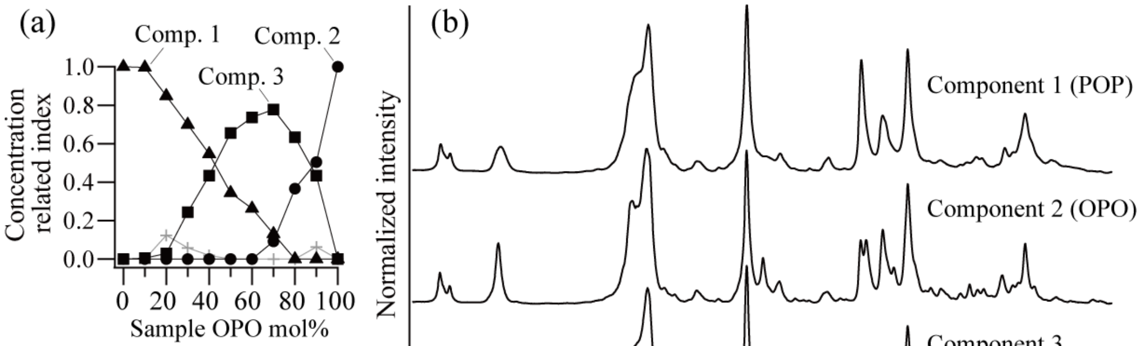

Fig. 2 Results of reconstruction (a) Concentration related index profiles. ▲, component 1; ●, component 2; ■, component 3; +, residuals. (b) Raman spectra of the components of POP-OPO binary system.

Chapter 5: TAG polymorphic phase behavior in biological systems

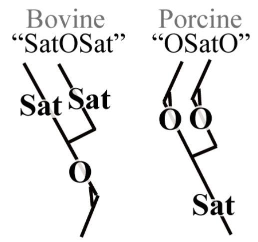

Fig. 3 Major TAG molecules in bovine and porcine fats.
Sat: Saturated acyl chain
O: Oleoly chain

It is empirically known that the mixing of multicomponent systems, accompanied by a large TAG compositional change, would indicate a transition to a completely different fat with different phase behavior. However, because of the complexity, the underlying causes for such transition are not known so far.

Adopting bovine and porcine fats as the instance of TAG multicomponent systems, the influence of the difference in TAG composition on their phase behavior and the phase behavior of their mixture are investigated. Bovine fats have high concentration of TAGs which have oleoyl acyls primarily substituted in their *sn*-2 position (Fig. 3). On the other hand, porcine fats have TAG molecules with oleoyls in their *sn*-1 and -3 positions.

Crystals of these two fats and their mixture fats were prepared by cooling down the melt to 0°C and holding for 5 min. Raman spectra of the polycrystal were measured by a 785-nm Raman spectrometer developed in the laboratory. The samples were kept at 0°C during the measurements.

The porcine fats show a band at 1417 cm^{-1} (Fig. 4), while the bovine fats do not exhibit this band.[2] This band is assigned to the CH_2-scissors mode characteristic of the orthorhombic subcell structure of β'-polymorph. It is therefore shown that the porcine fats contain the β'-polymorph while bovine fats do not contain them. The difference arises due to the TAG compositional difference between the two fats. The major TAG species in porcine fats (OSatO) is likely to form β'-polymorphs in the present experimental conditions.

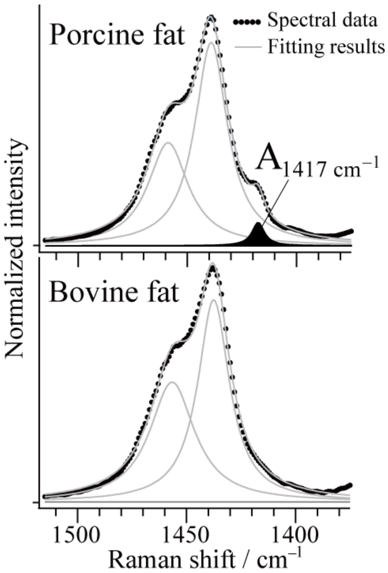

Fig. 4 Raman spectra of porcine and bovine fats.[2]

In bovine-porcine mixture systems, however, β'-polymorphs scarcely exist even in the presence of porcine fat upto 50% (Fig. 5). The SatOSat-OSatO type molecular compound formation is the most likely reason why the addition of the bovine fat disturbs the β'-polymorph formation. The empirically known drastic changes of phase behavior which are caused by mixing multicomponent systems probably arise due to molecular compound formation.

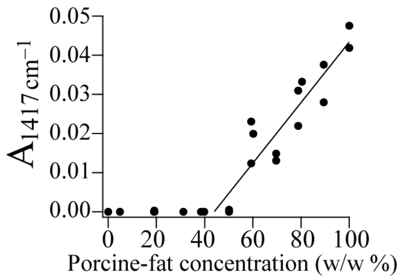

Fig. 5 Relation between $A_{1417\ cm^{-1}}$ and porcine-fat concentration.[2]

Chapter 6: Conclusion

By the use of Raman spectroscopy, the characterization of TAG phases in multicomponent systems has been conducted. In the POP-OPO binary system, the formation of the third component has been shown. In the biological bovine-porcine system, the molecular compound formation is suggested. It has been shown that Raman spectroscopy is the powerful tool to study about the phase behavior of multicomponent TAG systems. Future prospects of studies on TAG structural chemistry through the application of Raman spectroscopy have also been discussed.

1. Minato A, Ueno S, Yano J, Smith K, Seto H, Amemiya Y, Sato K *Journal of the American Oil Chemists Society* **1997**, *74*, 1213-1220.
2. Motoyama M, Ando M, Sasaki K, Hamaguchi H *Applied Spectroscopy* **2010**, *64*, 1244-1250.