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Crystal Growth of Triglycerides in Hydrogenated Oil

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(Figs. 1 ~ 8)

1. INTRODUCTION

The polymorphic crystal transformation of triglycerides consisting hydrogenated oils has been studied by E.S, Lutton[1] and T.Malkin[2] and C.W.Hoerr.[3] However, electron microscopic and X-ray diffraction studies both have never reported on the relation between the feature of crystal and its polymorphism.

It is well known from X-ray studies[1),2)] that there exist three or four modification polymorphism of triglycerides. We have already reported that the relations between crystal features of triglycerides and their transformations can be confirmed by electron microscopy.[4]

This is the case of triglycerides of simple saturated fatty acids, that is to say, consisting of tristearin and tripalmitin. However, triglycerides of fatty acid not only contain saturated fatty acid but also unsaturated carbon-carbon in double or triple bonds in many cases. Therefore, it is difficult to study their crystal morphology by electron microscope and to observe their fine structures. Such a study has not yet been reported so far.

The purpose of this report is to examine microscopically the hydrogenated oil crystals wich consist of triglycerides with low melting point, and to determine the polymorphism of crystals by X-ray diffraction. The interrelation of these two phenomena was clarified, the effect of the transformation rate on the crystal growth was examined, and the relation between crystal habit coarsness of crystal surface was also experimentally determined.

According to these experimental results, it was concluded that the time required for the crystalline transformation of hydrogenated oil is affecting the resultant crystal features. The results of the examinations of the dominant factors of such effects are also reported.

2. EXPERIMENTAL PROCEDURES

2.1. Specimen

Vegetable and animal oils were used. Both oils were hydrogenated; the former contain relatively simple kinds of fatty acids, while the latter ones contain more different kinds of fatty acids.

The vegetable oils used were hydrogenated soybeen oil, hydrogenated palm kernel oil and hydrogenated cotton seed oil. The animal oils were lard, tallow and hydrogenated lard.

In the case of vegetable oils, the crystal growth was difficult to carry out with crude oils themselves, owing to their low melting points. Thus their melting points were raised by the hydrogenation and then the suitable specimen for the crystal growth were obtained. The melting points of both vegetable and animal oils have been distributed between 30°C and 40°C.

The experiments of crystal growth have been done thus; firstly all specimen were completely melted at 60°C, and then rapidly cooled off onto 0°C, and kept at about 8°C for more than two weeks in order to perform enough annealing. The specimen obtained under such conditions were used in the following experiments.

2.2 Determination of polymorphic modifications

Polymorphic modifications were determined by X-ray diffraction. In order to measure the short spacing of unit cells of triglycerides, the angle from 19° to 25° was scanned by using a goniometer.

The forms of polymorphic modification were determined irrespective of the kinds of triglycerides from the characteristic diffraction patterns.

2.3. Preparation of specimen for electron microscopy

One stage atomic replica was adopted at room temperature for triglycerides containing only saturated fatty acids, while a special apparatus had to be used from those containing unsaturated fatty acids. The apparatus has already been described elsewhere[5], but yet the one used in this present work was partly modified as shown in Fig. 1. This is an apparatus for the low temperature replica method, and it is constructed to keep the specimen at low temperature during the preparation of replica in order to avoid the thermal effect. The temperature of the specimen was kept at about −150°C during vacuum evaporation. Germanium was used as a shadowing metal, and carbon was used as a reinforcement. Liquid nitrogen was used as a freezing mixture, and it was put in D in Fig. 1 to cool off the specimen. When liquid nitrogen was put in, the order of vacuum was thought to be adequate in the neighbourfood of the emission of the luminescence from a Geisler Tube. If this order of vacuum was not attained, the moisture in surrounding air should affect the state of surfaces of the specimen, thus the freezing mixture could not be used under several mm Hg at the dark part. After the replica was obtained by such a vacuum deposition, oil was dissolved with the mixed solution of aceton and benzen.

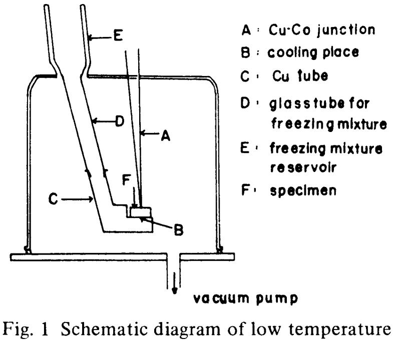

Fig. 1 Schematic diagram of low temperature replica apparatus

As the replica was separated before oil was dissolved into the solvent completely, it was put into the fresh solvent again in order to get a clear film. If any specimen were left in the replica, a good contrast of observed image could not be obtained, because such specimen should be carbonized as a result of the bombardment of electron beams. After the replica became clear, it was used in the observation by mounting on a mesh.

3. POLYMORPHIC TRANSFORMATIONS OF TRIGLYCERIDES CRYSTALS

The same specimen used in electron microscopy were employed for X-ray diffractometry. As it is shown in Fig. 2, triglycerides of vegetable oils were all β'-form and all of the other animal oils were β-form. The pattern (1) shows the superimposed figure of hydrogenated and unhydrogenated lard. The diffraction patterns of both completely coincide with them. The diffraction pattern of tallow (2), and the diffraction line at 3.85 Å are weaker than that of lard but two additional lines are observed. The reason for defining the modification of tallow as β-form is explained by its sharp and strong diffraction line observed at 4.57 Å, which has been proved to be characterestic one of β-form. The diffraction lines of β-form are the strong ones at 4.2 Å and 3.80 Å.

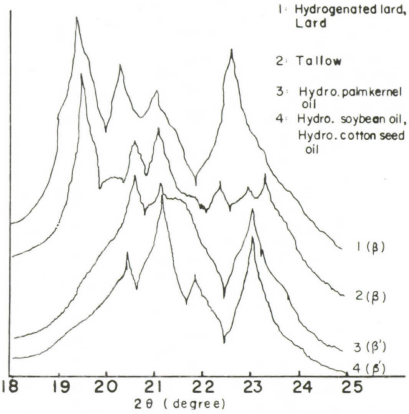

Fig. 2 X-ray diffraction pattern of polymorphic modification

Consequently the diffraction patterns of hydrogenated palm kerner oil (3), which is one of the vegetable oil, belong to β'-form as shown in (4).

For the transformation of polymorphism of triglycerides of vegetable and animal oils, each time required for the transformation to a stable modification from an unstable one is qualitatively slower in the former case and faster in the latter, respectively. The β-form modification, however, is supposed to become a stable form as a result of long annealing.

4. GROWTH MORPHOLOGY OF TRIGLYCERIDE CRYSTAL

From the results of electron microscopic studies of replicas prepared by the low temperature replica method, it was found that triglycerides of vegetable and animal oils were observed to grow into single crystals. The surface structurers of these single crystals can be roughly devided into two types, which are those of lamellar growth and spiral growth. However, the regularity in growth shapes is not found in all cases. Fig. 3 shows the crystals of hydrogenated soybean oil, in which grain-like crystals grow on the surface. Relatively small single crystals exist in the region without the grain-like crystals. The crystals for lamellar growth are about 1μ and grain-like crystals are between about 4μ to 8μ in dimensions. D.R.Merker et al.[6] studied cotton seed hydrogenated oil and soybean hydrogenated oil from a view point of polymorphism, and they found that grain-like crystals can grow macroscopically in soybean hydrogenated oil after a operation of annealing. It is β-form modification with high melting point resulting from the transformation of α-form as a result of the effect with the passage of time.

However, when cotton seed hydrogenated oil is added in an amount of more than 20%, this grain-like crystal forming is not found to occur. This is accounted for by the crystals having a stabilized β-form. These grain like crystals of between about 4μ to 8μ in dimensions are supposed to become nuclei at the stage of the macroscopic grain growth. Fig. 4 shows crystals of hydrogenated kerner oil, in which it is shown that their surfaces are not flat because spiral patterns are observed on the surfaces of the as-grown crystals. These single crystals grow spirally and are about 2μ in dimensions. Some of them grow to about 5μ. The shapes of these crystals are generally rectangular. The crystal growth with regular shape may occur too. The lamellar growth is observed only, sometimes generally they show spiral growths. Fig. 5 shows the crystals of hydrogenated cotton seed oil, in which spirally grown single crystals are seen in hydrogenated palm kerner oil. Their surfaces are, in cotrast to those of palm kerner oil, so flat that any roughness can hardly be observed. These single crystals are between about 1μ to 2μ in dimensions.

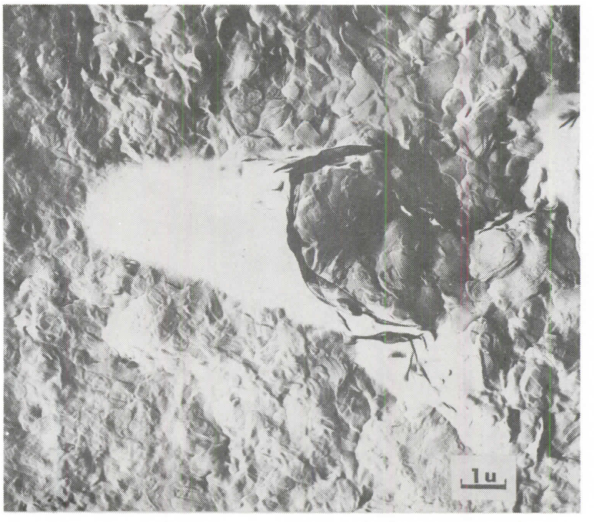

Fig. 3 Electron micrography of hydrogenated oil grain like crystal in hydrogenated soybean oil

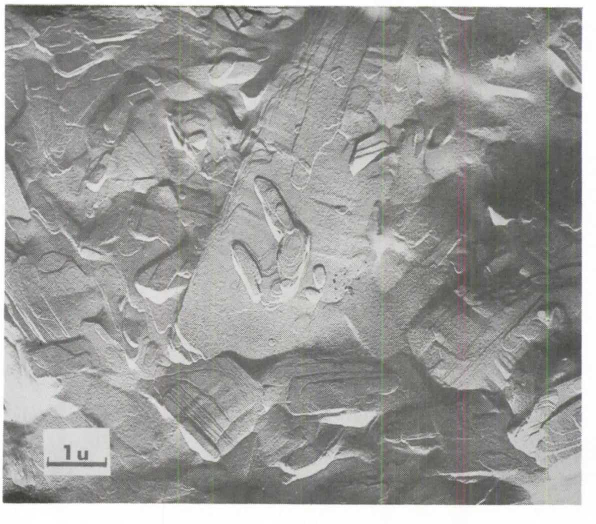

Fig. 4 Electron micrography of spiral growth of hydrogenated palm kernel oil crystal

The spirally grown single crystals observed in the center region of Fig. 5 are supposed to be grown from two nuclei. This characterestic growth mechanism thus; growth starts from the nucleus as the growth center, then the area of the terrace between each spiral step becomes larger in proportion to the crystal growth.

This is a remarkable feature of the crystal growth from melt in contrast to that from gas or solution. In other words, the number of molecules contacting the crystalline solid at the interface is remarkably larger in a molten state than in gas or in solution state. Therefore the solid interfaces must be flat in the latter case and rough in the former case corresponding to the number of the growth units at the interface.

The mechanism of crystal growth of animal oils is supposed to be different from that of vegetable oils. Fig. 6 shows the growth patterns of unhydrogenated lard crystals. Their surfaces are flat compared to those of vegetable oils. These single crystals are 2μ in dimensions. One of corners of each crystal has an acute angle but the other ones are round. Spiral or lamellar growth is not seen in the growth patterns of animal oils.

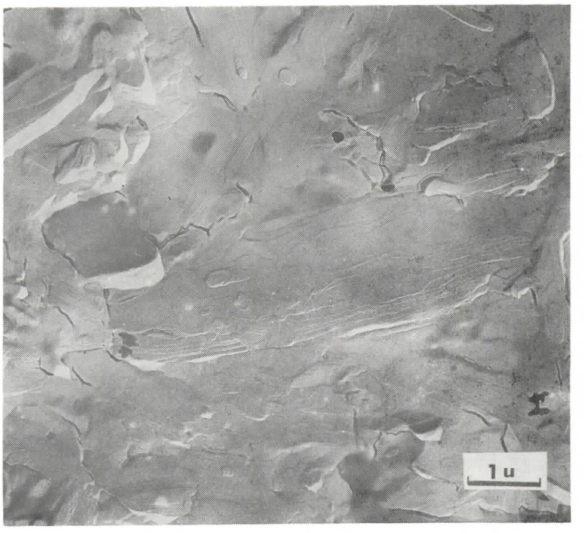

Fig. 5 Electron micrography of large spiral growth of hydrogenated cotton seed oil

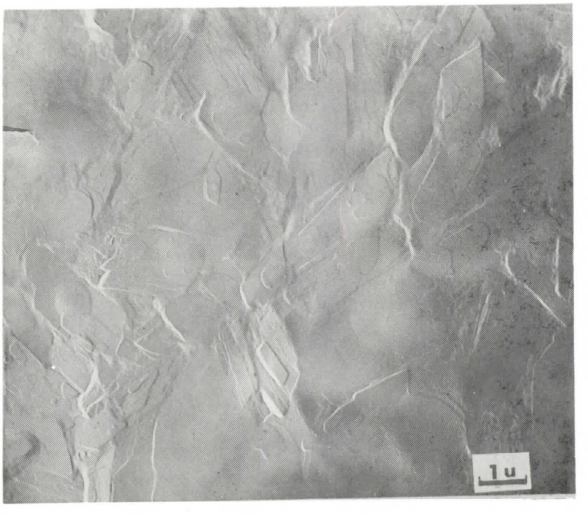

Fig. 6 Electron micrography of crystal flat thin single crystals of lard

The crystal growth of hydrogenated lard is shown in Fig. 7. the surfaces of this hydrogenated lard are relatively rougher than those of unhydrogenated lard shown in Fig. 6. Most shapes of these single crystals are irregular and partly rhombic shapes are observed in Fig. 7. Their sizes are about 3μ and it is supposed that the segregation has occurred. Fig. 8 shows the crystal growth patterns of tallow. These single crystals are rather slender compared to lamellar of lard and are about 3μ in length, 0.4μ in width. Some amorphous round matters are isolately observed, on which germanium shadows can not be detected. Germanium shadow appears at the step at the end of the terrace, the height of which is corresponding to the unit cell of triglyceride multiplied by integral numbers. As a result the round matters are suggested to be amorphous. On these surfaces the packing of single crystals is coarse, but no more roughness is observed.

In order to study the difference of the growth patterns between hydrogenated tallow and lard, some factors should be considered. As far as the molecular structure, there is no remarkable difference between tallow and lard. Then the melting point of tallow is higher tthan that of lard. However, when lard was hydrogenated, the melting point of hydrogenated lard was increased and its value did not differ so much from those of both unhydrogenated and hydrogenated tallow. For this reason, it was difficult to distinguish these two hydrogenated oils. It is worth while investigating the observed remarkable difference between the growth patterns of two kinds of triglycerides. Electron microscopical study on crystal shape has showed that the crystal habits have their own characterestics and the transformation of morphism has different modifications.

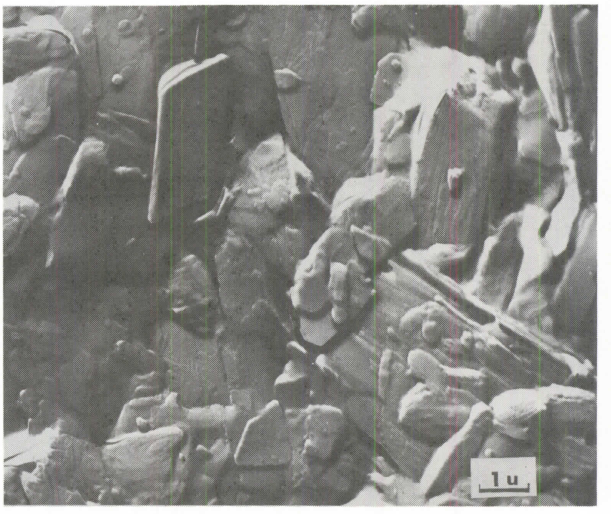

Fig. 7 Electron micrography of lamelar growth of hydrogenated lard

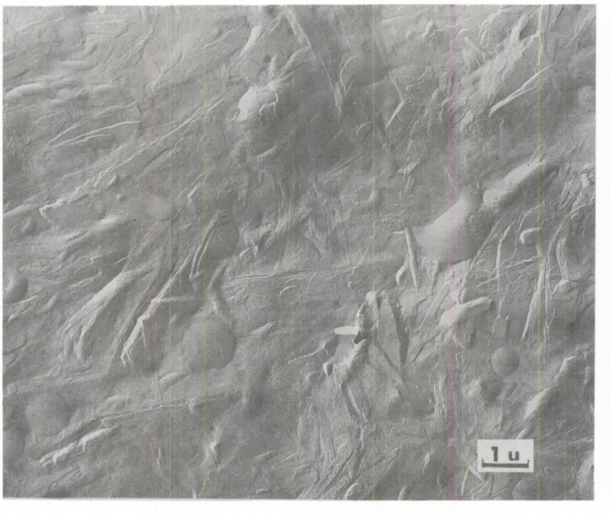

Fig. 8 Electron micrography of needle like crystals of lamellar growth of tallow

5. DISCUSSION

Some differences in shapes of triglyceride crystals grown from a molten state may appear according to the conditions of the crystal growth. These must be discussed in taking into account the transformations of modifications in polymorphism. As the transformations of modifications are accompanied soon after the crystal growth, the transformation is considered to have a dominant effect upon the crystal shape.

The modification in polymorphism corresponds to the transformation from the thermally unstable α-form unto stable β-form. The time required for the transformation to β-form depends on the chain lengths and the geometrical symmetries of saturated fatty acid molecules constituting triglyceride. When each molucular chain is highly symmetrical, short in length and the kinds of fatty acids included were few, the time required for the transformation is short. If not so, the time is long[7),8)]

The specimen used in these experiments were hydrogenated vegetable and animal oils. The former oils have the crystal structure of β-form and smaller shapes of molecules while the latter ones β-form and lager shapes. The time required for the transformation of β-form is longer than the time for β-form. In other words, after a given time, it happens that hydrogenated animal oils transform to stable β-form, while hydrogenated vegetable oils are transforming to β′-form but have not yet reached the stable modification phase completely. It is suggested that in the growth process of the β-form crystals the mean crystal shapes are small, as a result no large crystals appear. Because many crystal nuclei have appear in the earlier stage of the growth, the molecules as the growth units can adsorb to the nuclei and then the total adsorbed growth units over the growth surface may increase in number. So the rate of crystal growth be comes large. On the other hand, in the case of the crystal growth of β′-form, the number of crystal nuclei is less than of β′-form, the number of crystal nuclei is less than of β′-form, so the total number of the adsorbed growth units is less and the rate of crystal growth becomes small, and yet each single crystal will grow larger becouse of few nuclei and few total adsorbed molecules.

New let us discuss only the original effects of the hydrogenation of unsaturated fatty acids upon the rates of the crystal growth of animal and vegetable oils. As mentioned before in section 2, an animal oil contains complex kinds of unsaturated fatty acids while a vegetable oil simpler kinds of fatty acids. Therefore, it is more insufficient in an animal oil to perform complete hydrogenation of the unsaturated hydrocarbons than in a vegetable oil and it often happens that under the condition of the same degree of hydrogenation the unsaturared fatty acids still remain to some extent in the case of an animal oil. This is in our case. As a result of imperfect hydrogenation, liquid-state triglycerides, which are defined by the possibility of hard solidification, with remained unsaturated fatty acids tend to get mixed into hydrogenated oils during the process of the crystal growth in the case of an animal oil. This effect makes the rate of the crystal growth of an animal oil more larger than of a vegetable oil because of much number of adsorbable growth units in liquid-state triblycerides. Thus the result that the growth rate of an animal oil is larger than that of a vegetable oil corresponding to the effect of each crystal shape are further emphasized by the original effect of hydrogenation.

Until now it was believed that the factors dominating the rate of the crystal growth and the polymorphic transformation are the chain length of the molecule of fatty acid and its geometrical symmetry. However from the results of these studies ponnected to hydrogenation effect it is concluded that the existence of liquid-state triglycerides mixed within hydrogenated oils may be added into one of those dominant factors.

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水素添加油中でのトリグラセライドの結晶成長

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植物油および動物油に水素添加して，結晶性トリグリセライドの量を増加させて試料とし，それらの結晶形態が電子顕微鏡によって観察され，また，多形現象の相転移がX線回折によって測定された。これらの実験によって得られた結晶形態および多形現象の相転移について結晶成長機構を探る観点から検討するのがこの研究の目的である。

実験の結果は植物性油脂，動物性油脂のそれぞれの結晶変態が β'型，β型であり，これらの変態に対応する結晶形態が前者の場合比較的大きく，後者の場合小さい単結晶に成長することが見出された。

脂肪酸組成は植物性油脂が動物性油脂よりも脂肪酸の種類が少ない。その点から，植物性油脂変態の転移速度が早く，動物性油脂は遅いとされている。しかし実験結果は逆の傾向を示している。これは油脂を構成している液体トリグラセライドと結晶性トリグラセライドの重量比が，動物性油脂の方が植物性油脂よりも大きいために結晶性トリグラセライド分子が液体トリグラセライド分子を媒介にして容易に移動することができて単位格子を作り，また単位格子のパッキングを密にするため転移速度が早くなったと考えられる。

さらに結晶の形態において，β型よりβ'型の方が大きい単結晶に成長する機構は，液体トリグリセライド分子が多いため，結晶性トリグリセライド分子の移転が容易で，多くの結晶核ができやすくなり，単結晶は小さくなる。反対に結晶核が少ないと，核に吸着される結晶性トリグリセライド分子は多くなるので大きい単結晶が成長することになると思われる。