Crystallization of Fully Hydrogenated and Interesterified Fat and Vegetable Oil

Lu Zhang¹, Hisako Muramoto², Satoru Ueno²* and Kiyotaka Sato²

¹ School of Food and Biological Engineering, Zhengzhou University of Light Industry (Dongfeng Road 5#, Zhengzhou, Henan 450002, P.R.CHINA)
² Graduate School of Biosphere Sciences, Hiroshima University (1-4-4 Kagamiyama, Higashi-Hiroshima 739-8528, JAPAN)

Abstract: Owing to public concern regarding the adverse health effects of trans fatty acids, an alternative technology to trans fats has recently become an important issue. The interesterification of fully hydrogenated vegetable oil and liquid oil blends is one of the most versatile options. This paper reports a physical analysis of high-melting fat (HMF) prepared through the interesterification of fully hydrogenated soybean oil and regular soybean oil, and through fractionation. The thermal and structural properties of the HMF blended with salad oil at a mass ratio of 4:1 (called the HMF blend, hereafter), which was prepared as a model fat blend for margarine, were assessed using X-ray diffraction (XRD), differential scanning calorimetry (DSC), and polarized light microscopy (POM). To observe the polymorphic transformation, all samples were aged after crystallization, and the development of granular crystals during the aging process was observed. We found that the granular crystals are made of SOS/SSO, POS/PSO, and (SOS+POS)/(SSO+PSO) molecular compounds, all of which easily transform into β form with a double-chain-length structure.

Key words: crystallization, trans fats alternatives, DSC, X-ray diffraction, crystal morphology

1 INTRODUCTION

Many researchers have reported that dietary trans fatty acids raise low-density lipoprotein cholesterol levels in the blood, thereby increasing the risk of coronary heart disease¹. Due to concerns regarding the adverse health effects of trans fatty acids, new rules for fat labeling have been introduced in many countries². Therefore, scientists and food product manufacturers are extensively working on alternatives to trans fats³.

There are two primary reasons to use high-melting fats instead of oil: their oxidative stability and solid fat functionality. The hardness of a fat imparts certain desirable properties (e.g., crispness, snap, and texture) in food products⁴. For this reason, partially hydrogenated fats have been employed in margarine and shortening as high-melting fats. However, the partial hydrogenation of vegetable oil is a major source of trans fatty acids in food products¹,². Therefore, a technology for preparing solid fats containing low trans fats is crucial. The alternative trans fat technology detailed in this paper involves the interesterification of fully hydrogenated vegetable oil and liquid oil, the fractionation of semi-solid vegetable oils such as palm oil, and the utilization of emulsifier and waxes⁵. However, many problems have been encountered in controlling fat crystal structures (e.g., granular crystal formation)⁶. Such problems do not occur in partially hydrogenated fats, since the crystal formed is β -tending, and tiny crystals creating a fine crystal network are easily formed. Therefore, many technological studies have been conducted on alternative trans fat technologies that focus on crystal network formation and stabilization against granular formations.

Crystallization profoundly influences the final structures of fats and is intrinsically related to their rheological properties⁷.⁸. Thus, a study on the crystallization kinetics of interesterified fats is an important endeavor. Additionally, when the triacylglycerol composition of a given oil or fat is subject to change through interesterification, conjoined alterations in the thermal profiles are observed.

Several studies have focused on the influence of interesterification on the thermal and structural properties of fully hydrogenated vegetable oil⁹.¹⁰¹¹. The physical and rheological properties of palm oil-soybean oil and lard-canola oil systems after blending and chemical interesterification were evaluated by Rousseau and Marangoni et al.¹². The formation of β polymorphs was induced in these two systems. Ribeiro et al. evaluated the influence of chemical interesterification on the polymorphism and crystallization of canola oil and fully hydrogenated cottonseed oil blends,
demonstrating that randomization does not change the original crystalline polymorphism, and that the original and interesterified blends have a significant predominance of β' polymorphs\[^{19}\].

Aiming at the use of interesterified fats for food product applications, we examined the physical properties of HMF. The HMF was prepared through the interesterification of fully hydrogenated soybean oil and regular soybean oil, and further subjected to fractionation in order to separate the high- and low-melting fractions, the low-melting fractions was used as the final HMF. As a model fat for margarine, we prepared a fat blend of HMF:salad oil = 4:1 (w/w) (called the HMF blend, hereafter). The crystal morphology was observed by means of polarized light microscopy (POM) (Olympus CX31 PF optical microscope, Olympus Optical Co., Ltd., Japan). A small amount of the sample containing crystals was placed on a glass microscope slide and gently covered with a glass cover slip. Digital images were acquired using an Olympus DP12-2 camera (Olympus Corporation, Japan) with polarized light and amplified 20, 40, and 100 times.

\[\lambda = 0.154\text{nm}\]

2 EXPERIMENTAL PROCEDURES

2.1 Materials

A sample of HMF, which was prepared through the interesterification of fully hydrogenated soybean oil and regular soybean oil, and further subjected to fractionation, was kindly provided by Fuji Oil Co., Ltd. The model fat blend used for margarine was a blend of HMF and salad oil at a ratio of 4:1 (w/w). To obtain the most stable forms, thermodynamic equilibration was achieved by aging the crystallized mixture samples at 20°C for at least one week.

2.2 Methods

The transformation and melting of the HMF and HMF blend were measured using differential scanning calorimetry (DSC-8240, Rigaku Co., Tokyo, Japan) at rates of 2°C/min and 5°C/min, respectively. The crystal forms were measured using X-ray diffraction (XRD), DSC, and optical microscopy. The key properties assessed in this present study include crystal morphology, polymorphic behavior, and crystallization kinetics.

2.3 X-ray diffraction analysis

The transformation and melting of the HMF and HMF blend were measured using differential scanning calorimetry (DSC-8240, Rigaku Co., Tokyo, Japan) at rates of 2°C/min and 5°C/min, respectively. The crystallization and melting temperatures were 26.7°C and 23.8°C for the HMF and HMF blend, respectively, while the crystallization enthalpy values were 53.3 J/g and 40.1 J/g. Correspondingly, the melting point of the HMF decreased from 20.7°C to 17.3°C when blended with the salad oil (Fig. 1(B)). Blending with 20% liquid salad oil led to a 3°C decrease in the crystallization and melting points.

3 RESULTS AND DISCUSSION

3.1 Triacylglycerol Composition

The major triacylglycerol compositions of the fully hydrogenated and interesterified soybean oils were 17% SSS, 3% PSS, 2% SPS, 21% SOS, 11% SOS, 2% POS, and 4% PSO. After fractionation, the major triacylglycerol compositions changed to 1% SSS, 34% SSO, 17% SOS, 4% POS, and 7% PSO. The major high-melting TAGs of this HMF are SSO, SOS, POS, and PSO.

3.2 Thermal characteristics

Heating and cooling DSC thermograms of the HMF and HMF blend are depicted in Fig. 1. The temperature program consisted of storage at 50°C for 5 min, cooling from 50°C to −20°C (2°C/min), isothermal treatment at -20°C for 5 min, and then heating from −20°C to 50°C (2°C/min). The crystallization temperatures were 26.7°C and 23.8°C for the HMF and HMF blend, respectively, while the crystallization enthalpy values were 53.3 J/g and 40.1 J/g. Correspondingly, the melting point of the HMF decreased from 20.7°C to 17.3°C when blended with the salad oil (Fig. 1(B)). Blending with 20% liquid salad oil led to a 3°C decrease in the crystallization and melting points.

3.3 X-ray diffraction analysis

The HMF and HMF blend were crystallized from 50°C to 0°C at cooling rates of 0.1°C/min and 5°C/min, respectively. Figure 2 shows the XRD patterns of the HMF and HMF blend obtained at 0°C by a rotator-anode X-ray beam generator. With cooling at 5°C/min (Fig. 2(B)), the small-angle...
Crystallization of Hydrogenated and Interesterified Fat and Oil


Fig. 2 XRD patterns of the HMF and HMF blend crystals: (A) through slow cooling from 60°C to 5°C at a rate of 0.1°C/min, and (B) through rapid cooling from 50°C to 0°C at a rate of 5°C/min.

diffraction patterns of 4.90 and 2.45 nm correspond to the 001 and 002 reflections of the long spacing values of 4.90 nm of a double-chain-length structure. Wide-angle X-ray diffraction (WAXD) patterns of 2θ = 21.3° and 23.2° indicate lattice spacings of 0.42 and 0.38 nm, respectively. These patterns are characteristic of a sub-α form. Except for a minor difference in the long spacing value of 5.2 nm, the same pattern was observed for the HMF blend. Although not shown here, by decreasing the cooling rate to 2°C/min, the XRD patterns of the same polymorph of the sub-α were also observed. At a low cooling rate of 0.1°C/min (Fig. 2 (A)), one more WAXD reflection of 2θ = 19.4° appeared, corresponding to a lattice spacing of 0.46 nm, which is characteristic of the β form. The XRD patterns of sub-α form disappeared when the temperature was raised to 30°C, where a β polymorph coexists with a liquid line. The SAXD patterns clearly indicate a double-chain-length structure for each polymorph (4.9 nm for sub-α form and 4.4 nm for β form). As reported by Sato et al.15, SSO and SOS have triple-chain-length structures in all polymorphs except αSSO. However, although SOS and SSO are the two major TAGs and account for half of the total TAGs in the HMF, no polymorph with a triple-chain-length structure exists.

Regarding the SOS/SSO binary mixture, Takeuchi et al. reported a molecular compound in which α, β, and β polymorphs occur at a ratio of 50:50. All polymorphs of the SOS/SSO molecular compound were arranged in double-chain-length structures with a length of 5.46 nm for α, 5.00 nm for βL, and 4.56 nm for β. We assumed that the molecular compound formation also exists in both the HMF and HMF blend systems. The following discussion on the results of the most thermodynamically stable form will further elaborate this point.

To obtain the most stable polymorph, the HMF was aged at 20°C, and the HMF blend was aged at 10°C soon after rapid cooling. DSC and XRD data were collected each day to observe the changes in the polymorphs. One wide endothermic peak appeared during heating (Fig. 4). The melting point of the HMF increased from 32.2°C to 35.7°C; the peak top temperature increased from 35.6°C to 37.4°C during the nine-day aging period. For the HMF blend, the melting point and peak top temperature were 32.1°C and 35.2°C on the first day, and 32.9°C and 35.4°C on the ninth day, and only subtle changes were observed during the aging period.

Fig. 3 XRD patterns of the HMF and HMF blend at different temperatures while heating from -20°C to 50°C after cooling and isothermal treatment.

3.4 Polymorphism

The HMF blend was crystallized from 60°C to -20°C at a cooling rate of 2°C/min, maintained at this temperature for 5 min, and then heated at 2°C/min. During this temperature variation, small angle X-ray diffraction (SAXD) and WAXD patterns were obtained (Fig. 3). Sub-α form, which has the same XRD pattern as that shown in Fig. 2 (B), was observed after cooling. After heating to 0°C, a WAXD peak of 0.46 nm, corresponding to a β polymorph, appeared. Further heating increased the WAXD intensity of the diffraction peak at 0.46 nm, and decreased the intensity of the WAXD peaks at 0.416 nm and 0.384 nm (sub-α). The XRD patterns of sub-α form disappeared when the temperature was raised to 30°C, where a β polymorph coexists with a liquid line. The SAXD patterns clearly indicate a double-chain-length structure for each polymorph (4.9 nm for sub-α form and 4.4 nm for β form). As reported by Sato et al.15, SSO and SOS have triple-chain-length structures in all polymorphs except αSSO. However, although SOS and SSO are the two major TAGs and account for half of the total TAGs in the HMF, no polymorph with a triple-chain-length structure exists.

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The XRD patterns of the HMF blend (Fig. 5) indicate that only a β polymorph existed. These results indicate that the unstable sub-α form transformed into a β form very quickly, and that the transformation was complete within a single day. Blending the HMF with low-melting-temperature salad oil promoted the formation of a stable β polymorph. Based on the DSC data, we speculate that the wide endothermic peak (Fig. 4) included not only SOS/SSO molecular compounds in the β polymorph, but also other β-form molecular compounds. The occurring molecular interactions, which mostly influence the stabilization of the crystal structures of TAGs containing saturated and unsaturated fatty acid chains, are aliphatic chain packing, glycerol conformation, and methyl end stacking. Molecular compound formations, were observed in the binary mixture systems of the symmetric/asymmetric saturated/unsaturated mixed-acid TAGs, such as POP/PPO and SOS/SSO. The mechanism of such molecular compound formation was discussed in a study on POP/OOP binary systems, in which a eutectic phase was formed.

In the HMF and HMF blend, we can assume that several molecular compound crystals can be formed in the SOS/SSO, POS/PSO, and (SOS + POS)/(PSO + SSO) mixtures (Fig. 6).
To compare the fat crystal textures of the HMF and HMF blend formed at different cooling rates, optical observations were conducted before and after aging of the HMF and HMF blend crystallized through rapid ($\frac{5}{1}$C/min) and slow cooling ($\frac{0.1}{1}$C/min) (Figs. 8 and 9).

For the HMF and HMF blend formed through slow cooling (Figs. 8B and 9B), the fats showed crystals with larger diameters than those produced through rapid cooling (Figs. 8A and 9A). The diameters of the crystals increased when the HMF was blended with salad oil (Fig. 9), although the crystal morphology was not altered by the dilution. A subtle growth in the granular crystals observed by POM indicated that the texture of the fat became compacted due to the aging process (Figs. 8C, 8D, 9C, and 9D).

4 CONCLUSIONS

The major high-melting TAGs of the HMF are 1% SSS, 34% SSO, 17% SOS, 4% POS, and 7% PSO. Blending with liquid oil (salad oil) decreased the crystallization and melting temperatures. Both the HMF and HMF blend, aged after crystallization, rapidly transformed into $\beta$ form and then developed into granular crystals. Additionally, blending with salad oil promoted a significant increase in crystal size. Thermal and structural analyses indicated that the granular crystals are made of molecular compounds of SOS/SSO, POS/PSO, and $(SOS + POS)/(SSO + PSO)$, all of which easily transformed into $\beta$ form with a double-chain-length structure.

References

