Effects of Ultrasonic Irradiation on Crystallization Behavior of Tripalmitoylglycerol and Cocoa Butter

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ABSTRACT: Effects of application of ultrasonic power (20 kHz, 100 W) on the crystallization behavior of tripalmitoylglycerol (PPP) and cocoa butter have been examined in terms of rate of nucleation and polymorphic control. High-purity PPP (>99%) and low-purity PPP (>80%) samples were employed to mimic real fat systems, which usually have higher concentrations of minor components in addition to the main component. For both the high-purity and low-purity PPP, the application of ultrasonic power accelerated the rate of nucleation as measured by induction time for the occurrence of crystals and by the number of crystals nucleated. As for the polymorphic influences, the nucleation of both the β′ and β forms was accelerated by the ultrasound, yet the β′ form nucleation was more accelerated when the low-purity PPP samples were employed. As for cocoa butter, sonication for a short period accelerated the crystallization of Form V. The present results indicate that ultrasound irradiation is an efficient tool for controlling polymorphic crystallization of fats.

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KEY WORDS: Cocoa butter, fat crystallization, polymorphism, sono-crystallization, tripalmitoylglycerol, ultrasound, X-ray diffraction.

Fats are employed in foods, cosmetics, and pharmaceuticals. They may be the end product, or they may be matrices in which cosmetic and pharmacological fine chemicals are dispersed (1). The crystallization behavior of fats has two major industrial implications: (i) processing of end products made of fat crystals, such as chocolate, margarine, shortening, and whipping cream, and (ii) separation of specific fat materials from natural resources (2). Resources of natural fats are vegetable and animal fats and oils that contain various molecular species having different chemical and physical properties. The following market demands have created an increasing necessity to develop the fractionation technology for high-melting and low-melting fats and lipids: (i) to obtain high-melting fats by dry fractionation instead of hydrogenation, which produces *trans*-fatty acids as by-products, (ii) to cope with new regulations regarding the use of fat materials for confectionery end products, and (iii) to maintain better functionality of physically refined vegetable oils compared to conventional materials.

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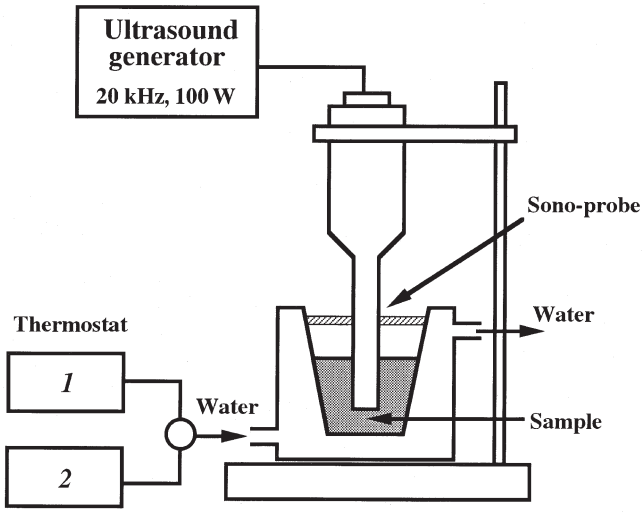
Ultrasound power has been thought to provide substantial opportunities for controlling the fat crystallization. In general, ultrasound at high power and low frequencies can assist various processes in food technology involving crystallization (3,4). As for the effects on crystallization, high power ultrasound (here referred to sono-crystallization) influences both nucleation and crystal growth remarkably, by creating new additional nucleation sites in the crystallization medium (5–8). Quite recently, examination of sono-crystallization in confectionery fats has indicated the possibility that tempering is achieved by sono-crystallization (9). However, the details of the sono-crystallization processes of fats are still open to question.

This paper presents experimental results on the effects of high-power (20 kHz; 100 and 300 W) ultrasound on the crystallization behavior of tripalmitoylglycerol (PPP) which was chosen as a model triacylglycerol (TAG) substance (10,11), and cocoa butter as a representative of confectionery fats (12). The rate of nucleation of polymorphic forms of the fats employed was studied by carefully controlling the temperature and duration of ultrasound application to liquidus fats prior to crystallization.

MATERIALS AND METHODS

Materials. High-purity PPP (purity >99%) and low-purity PPP (purity >80%) were purchased from Sigma Co. (St. Louis, MO). These were compared to elucidate the effects of the presence of minor TAG components. The TAG concentrations of the low-purity PPP were measured using gas chromatography under the following conditions: pretreatment by trimethylsilicate; CPSil 8CB (metal) columns; temperature program: hold at 75°C for 1 min, raise to 150°C (25°C/min) where the sample is held for 3 min, and raise (13°C/min) to 315°C, raise (1.5°C/min) to and hold at 355°C; flame-ionization detector. The weight percent composition of low-purity PPP was as follows: free fatty acids (FFA), myristic (0.57) and palmitic (1.16); diacylglycerol (DAG) dipalmitoylglycerol (PP: 4.07); and triacylglycerols PPP (80.73), 1-myristoyl-2,3-dipalmitoylglycerol (MPP: 3.32), 1-stearoyl-2,3-dipalmitoylglycerol (SPP: 9.73) and 1,2-distearoyl-3-palmitoylglycerol (SSP: 0.41).

Sono-crystallization. (i) PPP. Sono-crystallization was carried out with the equipment shown in Scheme 1. Sonication was done with a microcomputer-controlled ultrasound

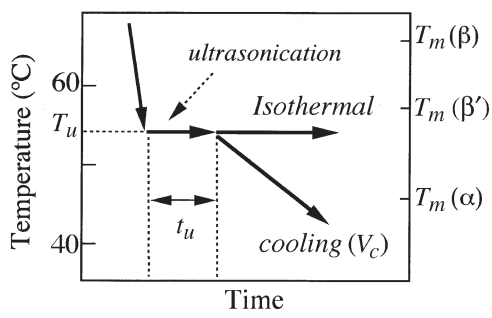


SCHEME 1

generator (model DG-100-20; Telsonic Co., Bronschhoften, Switzerland) with 20 kHz and 100 W. The PPP sample of 2 mL was set in a glass cell jacketed by circulating water connected to two thermostats: one for melting and the other for crystallization. The temperature of the sample was measured by thermocouple, with an accuracy of ±0.5°C. The duration of the sonication was usually 15 s. For comparison, a pulse-type sonication was performed with 3 s sonication and 3 s cessation, and stepwise sonication was made for 5, 10, 15, 30, 45, and 60 s. During sonication, cavitation occurred, and the sample temperature increased to about 80°C soon after the sonication was initiated.

Scheme 2 illustrates the temperatures of sonication and crystallization. The sample was cooled from 85°C to sonication temperature T_u; the time of sonication was t_u. Two modes of crystallization were employed after the sonication: isothermal crystallization was made at the temperature defined by T_u, and cooling crystallization was conducted after the sonication at various cooling rates defined as V_c.

The isothermal crystallization was used to observe the β′ and β forms for the high-purity and low-purity samples. By contrast, the occurrence of α and β′ forms was observed with the cooling crystallization. T_u was varied between the melting points of the β′ form (57°C) and the α form (45°C) of PPP. The sonication



SCHEME 2

probe (sono-probe) was thermally equilibrated with the sample at T_u before the sonication. V_c was 1, 2, and 4°C/min.

The isothermal crystallization was monitored *ex-situ* by a polarized optical microscope. Soon after the sonication was ended, a droplet of the PPP liquid (*ca.* 2.5×10^{-3} mL) was put in a growth cell whose temperature was controlled by thermostated water with accuracy of ±0.1°C. The growth cell was put on the stage of the optical microscope for observation of the formation of crystals. Induction time (τ) for the isothermal crystallization was defined as the duration until the first crystals were detectable. Polymorphic forms of the PPP crystals grown during isothermal crystallization were detected by X-ray diffraction. For cooling crystallization, optical observation was not applied, since the α and β′ forms appeared quite rapidly. Naked-eye observations were made, and the polymorphic forms of the crystallized PPP were detected by X-ray diffraction.

Cocoa butter. Ultrasound was applied to cocoa butter from the Ivory Coast, using a model DG-2000-2 ultrasonicator (Telsonic Co.) at 20 kHz and 300 W. The 250-mL sample was put in the temperature-controlled cell whose design was basically the same as shown in Scheme 1. Sonication was performed at 32.3°C for up to 15 s after cooling from 60°C. The sample temperature increased to 32.9°C as a result of sonication for 3 s, and to 36.2°C for 15 s. Ultrasound-treated samples were then aged at 20°C for 30 min and subjected to crystallization at 4°C.

The polymorphic forms of the crystallized PPP and cocoa butter samples were determined by X-ray diffraction [Rigaku (Tokyo, Japan) RINT-TTR (rotator-anode, Cu Kα, Ni-filtered, 40 kV, 250 mA)]. Two minutes after the crystallization processes, the sample was cooled and subjected to X-ray measurement at 20°C, so that the solid-state interconversion from one polymorph to other polymorphs was actually blocked. The relative concentrations of the polymorphic forms of the crystallized materials were calculated from intensity ratios of long-spacing and short-spacing X-ray diffraction spectra.

RESULTS

Temperature variation with sonication. Figure 1 shows the sonication time-temperature plot during the isothermal crystallization at 53ºC. As shown here, the sample temperature of PPP (2 mL, placed in the thermostated glass cell) rose by about 30ºC during the 10-s sonication. After sonication had ceased, the temperature dropped rapidly and then rose again owing to crystallization which occurred about 100 s after the sonication. The polymorph of the crystallized PPP was determined as β by X-ray diffraction (discussed shortly). This means that, even if the sample temperature exceeded the melting point of β (65ºC) as a result of the sonication, the crystallization was induced.

PPP. (i) High-purity samples. Figure 2 shows the rate of crystallization and polymorphic crystallization behavior of β and β′ of the PPP crystals subjected to sonication at different T_u values. The rate of crystallization is expressed by 1/τ, where τ is the induction time for the appearance of the first crystals during the isothermal crystallization.

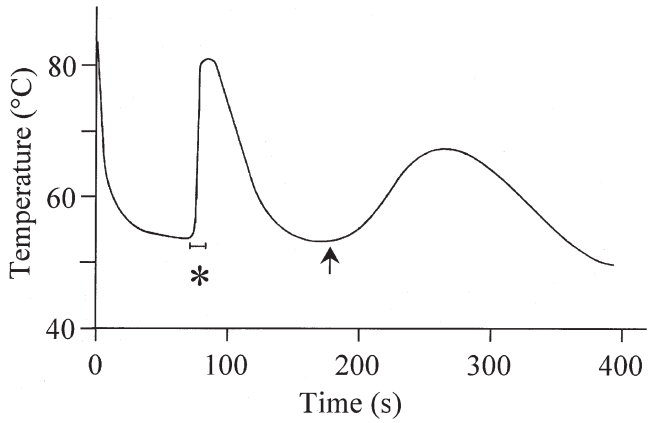


FIG. 1. Temperature-time profile during isothermal crystallization at 53°C under sonication. Sonication was carried out over 10 s (noted by asterisk), and the heat of crystallization was detectable at the time noted by the arrow.

For both polymorphs β and β′ and at all T_u, sonication decreased the 1/τ values, indicating the acceleration of the crystallization. For example at T_u = 46°C, τ was 80 s without sonication and 30 s with sonication for the β′ form; for the β form at 54°C, τ was 1740 s and 180 s without and with sonication, respectively. Furthermore, the occurrence of the β′ form was extended a bit toward higher T_u ranges by sonication. That is, no β′ form occurred at T_u = 52, 53, or 54°C without sonication, whereas crystallization of β′ was detectable at these temperatures with sonication, although the β form was also crystallized. The pattern of occurrence without sonication was the same as reported previously (10). The X-ray diffraction spectra of the PPP crystals obtained by the methods used to prepare Figure 2 are shown in Figure 3.

Optical photomicrographs of the β′ and β crystals obtained after isothermal crystallization at T_u = 48 and 54°C, respec-

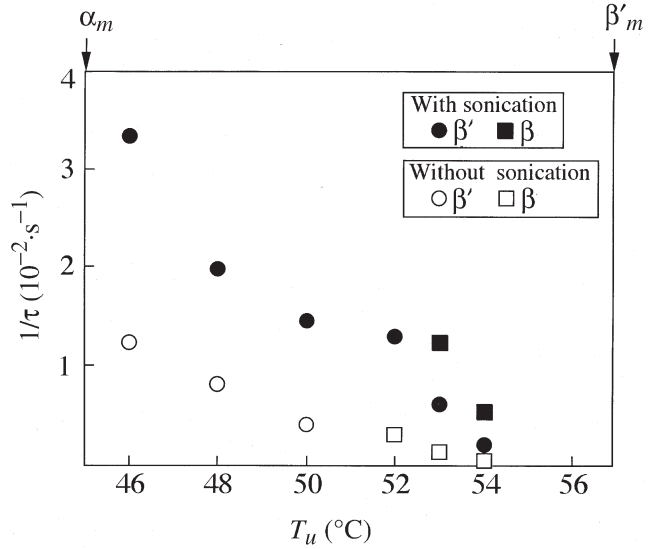


FIG. 2. Inverse induction time (τ) for melt crystallization of β′ and β polymorphs of tripalmitoylglycerol with and without sonication at different sonication temperatures (T_u). α_m and β'_m, melting temperatures of the α and β′ forms, respectively.

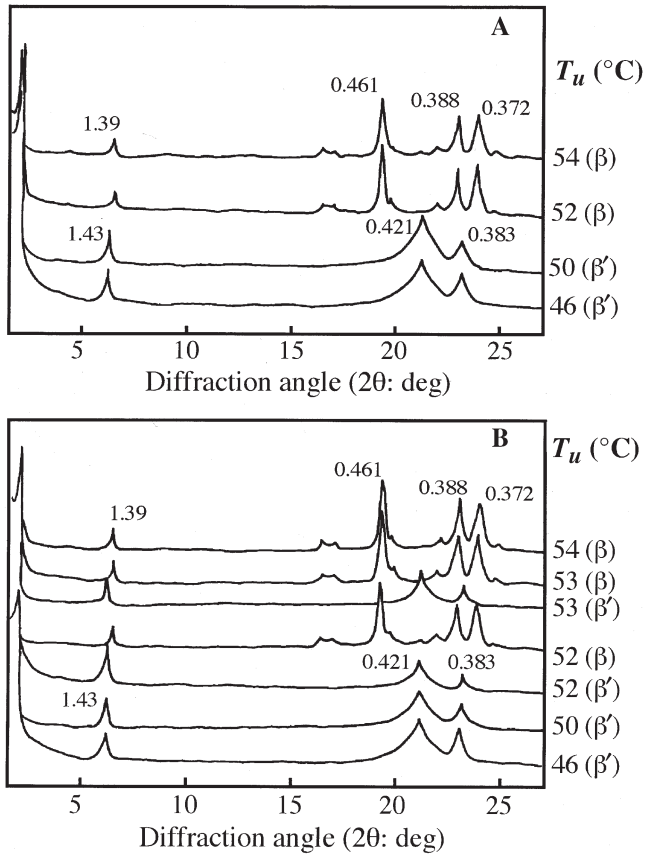


FIG. 3. X-ray diffraction spectra of high-purity tripalmitoylglycerol crystals obtained by isothermal crystallization: (A) without sonication and (B) with sonication. Units: nm.

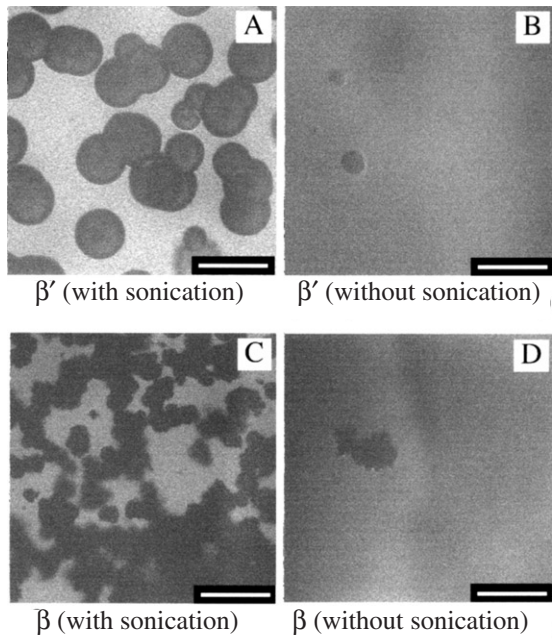


FIG. 4. Optical photomicrographs of polymorphic forms of tripalmitoylglycerol crystals obtained after isothermal crystallization from the melt with or without sonication. (A) and (B): β′ with and without sonication at 48°C, (C) and (D): β with and without sonication at 54°C , respectively. Scale bar: 200 μm.

tively, as shown in Figure 4. The polymorphic forms of the crystals shown by the photographs were examined by X-ray diffraction. Photomicrographs for the β′ form were taken 3 min after isothermal crystallization had been initiated, for the crystallization both with and without sonication (Figs. 4A,4B). For the β form, photomicrographs were taken after 3 min of sonication and at 30 min without sonication (Figs. 4C,4D). In Figure 4, the number of crystals nucleated by sonication was considerably increased for both β′ and β. In particular, β nucleation was highly accelerated by sonication; in the absence of sonication no crystals were detectable for at least 25 min. In light of the induction time results shown in Figure 2, one may conclude that the nucleation rate for high-purity PPP was accelerated by the ultrasound stimulation in terms of both the number of nucleated crystals and the induction time.

Low-purity samples. Figure 5 shows relative concentrations of the β′ and β crystals of the low-purity PPP samples obtained by isothermal crystallization at 52°C. Sonication was for 15 s. X-ray diffraction spectra specific to β′ and β were employed for determining the relative occurrence of the two forms. Although it was not dramatically visible, sonication increased the concentration of β′ compared to β. The induction times for the crystallization were also shortened by the sonication, both for β′ and β (data not shown).

The cooling crystallization of the low-purity PPP liquid was examined to observe the relative occurrence of the α and β′ forms after sonication by the two methods: variation in sonication time at a constant rate of cooling, and variation in cooling rate at a constant sonication time.

Figure 6 shows the X-ray results of the cooling crystallization with varying sonication time (t_u) at a constant rate of cooling (2°C/min). Sonication was done at 54°C. In Figure 6A, the α form was predominant at both shorter and longer t_u values, whereas the concentration of β′ was increased in a range of t_u from 5 to 30 s. In particular, the crystallization of the β′ form was most enhanced by sonication at 15 s. About 60–80% of the crystallized sample was of the β′ form, as shown in the relative concentration ratios calculated from the intensities of the specific X-ray diffraction long- and short-spacing spectra (Fig. 6B). Figure 7 shows the X-ray diffraction results from the cooling crystallization with varying cooling rate (V_c) with two

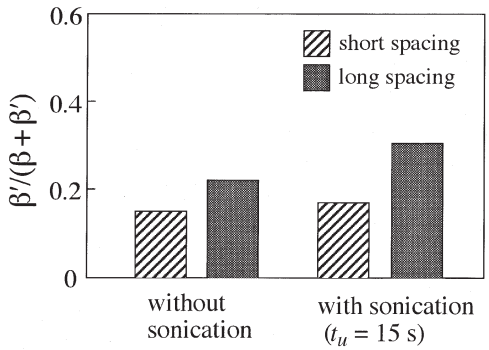


FIG. 5. Relative concentrations of β′ and β of low-purity tripalmitoylglycerol crystals obtained by isothermal crystallization (T_u = 52°C) with and without sonication.

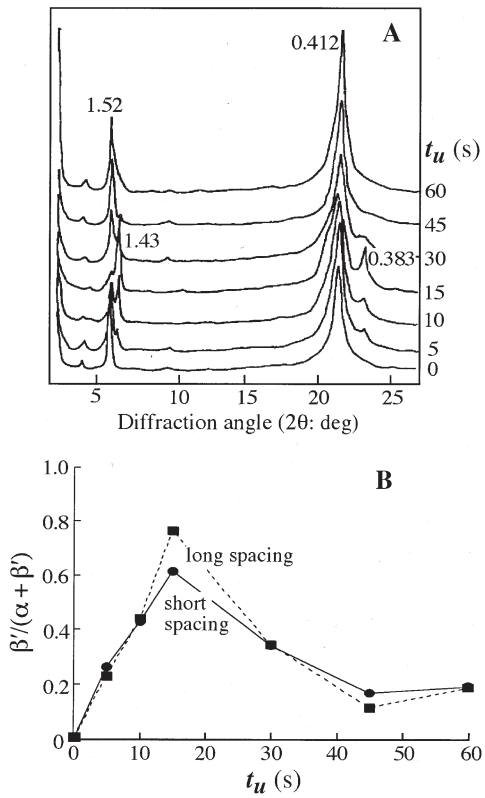


FIG. 6. Effects of sonication time (t_u) on polymorphic crystallization of α and β′ forms of low-purity tripalmitoylglycerol in cooling crystallization. (A) X-ray diffraction spectra (unit, nm), and (B) relative concentration of β′. T_u = 54°C and V_c = 2°C/min.

sonication times (t_u = 0 and 15 s). All experiments were performed at a constant temperature (T_u = 52°C). The long- and short-spacing X-ray diffraction spectra of the crystallized PPP at different t_u and V_c are shown in Figure 7A, and the relative occurrence of α and β′ are calculated from the diffraction spectra intensity in Figure 7B. Without sonication (t_u = 0), the concentration of β′ decreased with increasing V_c, and the α form was more crystallized. The same tendency was observed by following sonication for 15 s, when the concentration of β′ was decreased as V_c increased from 1 to 4°C/min, as shown in Figure 7B. Sonication in a pulse form did not vary the crystallization behavior of the β′ form.

Cocoa butter. Figure 8 shows that sono-crystallization induced the formation of Form V under limited conditions. Without sonication, Form II was crystallized (Fig. 8, line a), as in the case of cocoa butter crystallization without tempering. Sonication for 3 s produced Form V as shown in Figure 8, line b. The temperature of the cocoa butter sample rose by 0.6°C because of the transfer of mechanical energy into heat during the sonication. A temperature variation of 60.0/32.3/32.9°C does not correspond to the tempering. Therefore, the line representing the result (Fig. 10b) indicates that the sonication directly induced the formation of crystal nuclei of Form V in the liquid cocoa butter. Further treatment with ultrasound over 9 s resulted in the formation of a mixture of Forms II and V (Fig. 8, line c), and Form II was again

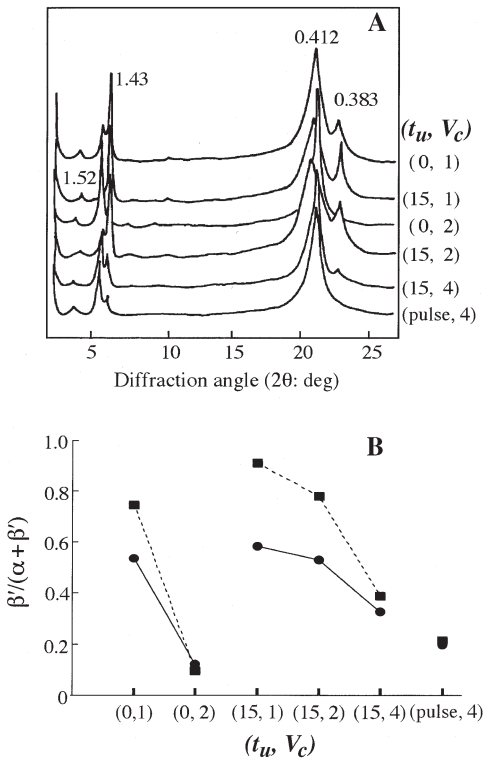


FIG. 7. Effects of sonication time (t_u, s) and cooling rate (V_c, °C/min) on polymorphic crystallization of α and β′ forms of low-purity tripalmitoylglycerol in cooling crystallization. (A) X-ray diffraction spectra (unit, nm), and (B) relative concentration of β′. T_u = 52°C. ■, long spacing; ●, short spacing.

crystallized by itself after sonication over 15 s (Fig. 8, line d). The temperature of the cocoa butter sample rose to 34.3 (9 s) and 36.2°C (15 s) with sonication. Since the melting point of Form V of cocoa butter is placed around 33–34°C, the rise in temperature produced by sonication may induce the melting of Form V crystal nuclei when sonication time exceeds 9 s. A long sonication time, 15 s, must completely melt Form V crystal nuclei. Thus the effect of sonication is diminished owing to the conversion from ultrasound mechanical energy to thermal energy.

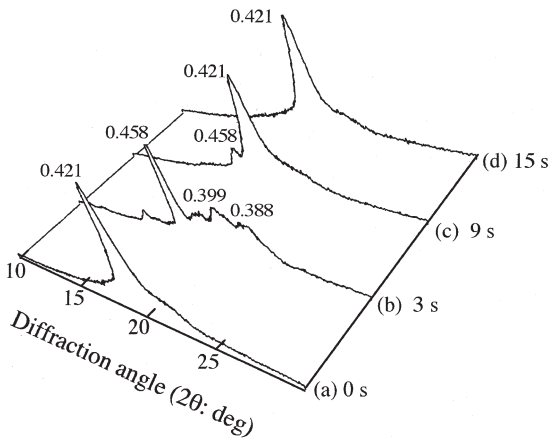


FIG. 8. X-ray diffraction spectra of cocoa butter, showing effects of length of sonication on polymorphic crystallization (unit, nm).

In the traditional tempering sequence, unstable Forms III or IV of cocoa butter are crystallized during the first cooling, and then the unstable forms transform to stable polymorphs during the reheating process. This polymorphic change occurs through melt-mediated transformation in which the stable Form V, having the triple chain length structure, is formed soon after the melting of the unstable forms having the double chain length structure. However, in the case of sono-crystallization, it is quite curious to see that the stable form is directly crystallized without the formation and subsequent melt-mediation of the unstable forms. The mechanisms underlying the polymorph-controlling sono-crystallization process are not clear. There would be some combined effects inherent to sono-crystallization, most probably due to pressure-induced thermodynamic effects and chain-chain interactions leading to the formation of stable molecular packing of triple chain length and the $T_{//}$ subcell of Form V.

DISCUSSION

The present experiments are briefly summarized in the following.

The application of ultrasound high power (sonication) increased the rate of crystallization of PPP polymorphs of β′ and β when sonication was performed prior to isothermal crystallization. The acceleration of crystallization seems to be revealed in the nucleation process, as exhibited in the shortened induction times (Fig. 2) and in the increase in the number of crystals (Fig. 4).

The cooling crystallization performed after sonication showed that certain optimal sonication conditions are determined by sonication time and temperature. For example, in the competitive crystallization of α and β′ forms of PPP, the sonication for 15 s at 54°C was optimal for the predominant crystallization of β′ at the expense of α (Fig. 6). Similarly, sonication for 3 s was best for the predominant crystallization of Form V against Form II in cocoa butter (Fig. 8).

Sonication increased the sample temperature due to its acoustic energy even above the melting point of the crystallizing polymorphic forms; e.g., the sample temperature of PPP soon reached about 80°C by sonication at 54°C, and the melting point of β′ is 57°C. Even so, β′ form crystallization was induced during the subsequent cooling when the sonication time was in the range of 5 to 30 s (Fig. 6).

These results strongly suggest that sonication is quite effective in increasing the crystallization rate and extent and in controlling the polymorphic crystallization.

In general, sonication affects the nucleation processes through the following mechanisms: (i) violent collapse of cavitation bubbles may form active sites for nucleation, (ii) enhanced agitation may effect profound mobility of crystallizing molecules, (iii) cooling caused by evaporation from the surface of the cavity during the growth of a cavitation bubble may increase supercooling, (iv) local pressure may increase the melting point in the vicinity of a collapsing cavity, which means that the degree of supercooling is increased. For

the moment, no single mechanism may explain the experimental results described above, and some combined mechanisms must be considered. In addition, geometrical interactions of the long-chain molecules of fats may also be important, since recent studies indicate that lamella formation is a prerequisite for three-dimensional nucleation of TAG (13–15). In this respect, there is another possibility: sonication may induce the formation of the lamella structure in liquid due to laminar flow caused by the collapse of the cavitation bubbles. Many of these mechanistic origins of the sonication effects on the crystallization of fats are open to question.

As for the possible effects of ultrasonication on heterogeneous nucleation involving polymorphic crystallization, heterogeneity may be revealed in the interactions with containers and dusts/impurities present in the neat liquid. In general, one may not exclude this possibility, because no experimental conditions without the containers and impurities are possible, and ultrasonic irradiation may violate the heterogeneous interactions of this kind. However, homogeneous interactions between triacylglycerol molecules possibly are stimulated by ultrasonication so that nucleation is promoted.

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REFERENCES

1. Gunstone, F.D., and F.B. Padley (eds.), *Lipid Technologies and Applications*, Marcel Dekker, New York, 1997.
2. Sato, K., Crystallization Behavior of Fats and Lipids—A Review, *Chem. Eng. Sci., 56/57*:2255–2265 (2001).
3. Mason, T.J., L. Paniwnyk, and J.P. Lorimer, The Uses of Ultrasound in Food Technology, *Ultrasonics Sonochem. 3*: S253–S260 (1996).
4. Mason, T.J., Power Ultrasound in Food Processing—The Way Forward, in *Ultrasound in Food Processing*, edited by M.J.W. Povey and T.J. Mason, Blackie Academic & Professional, London, 1998, pp. 105–126.
5. Hem, S.L., The Effect of Ultrasonic Vibrations on Crystallization Processes, *Ultrasonics*:202–207 (1967).
6. Enomoto, N., T.H. Sung, Z.E. Nakagawa, and S.C. Lee, Effect of Ultrasonic Waves on Crystallization from a Supersaturated Solution of Alum, *J. Mater. Sci. 27*:5239–5243 (1992).
7. Eskin, G.I., Influence of Cavitation Treatment of Melts on the Processes of Nucleation and Growth of Crystals During Solidification of Ingots and Castings from Light Alloys, *Ultrasonics Sonochem. 1*:S59–S63 (1994).
8. Ohsaka, K., and E.H. Trinh, Dynamic Nucleation of Ice Induced by a Single Stable Cavitation Bubble, *Appl. Phys. Lett. 73*: 129–131 (1998).
9. Baxter, J.F., G.J. Morris, and G. Gaim-Marsoner, Process for Retarding Fat Bloom in Fat-based Confectionery Masses, EU-Patent application: 95306833.5 (1995).
10. Sato, K., and T. Kuroda, Kinetics of Melt Crystallization and Transformation of Tripalmitin Polymorphs, *J. Am. Oil Chem. Soc. 64*:124–127 (1987).
11. Kellens, M., W. Meeussen, and H. Reynaers, Study of the Polymorphism and the Crystallization Kinetics of Tripalmitin: A Microscopic Approach, *Ibid. 69*:906–911 (1992).
12. Wille, R.L., and E.S. Lutton, Polymorphism of Cocoa Butter, *Ibid. 43*:491–496 (1966).
13. Ueno, S., A. Minato, H. Seto, Y. Amemiya, and K. Sato, Synchrotron Radiation X-ray Diffraction Study of Liquid Crystal Formation and Polymorphic Crystallization of SOS (*sn*-1,3-distearoyl-2-oleoyl glycerol), *J. Phys. Chem. B 101*:6847–6854 (1997).
14. Ueno, S., A. Minato, J. Yano, and K. Sato, Synchrotron Radiation X-ray Diffraction Study of Polymorphic Crystallization of SOS from Liquid Phase, *J. Cryst. Growth 198/199*:1326–1329 (1999).
15. Sato, K., Solidification and Phase Transformation Behavior of Food Fats—A Review, *Fett/Lipid 101*:467–474 (1999).

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