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Effects of Degree of Enzymatic Interesterification on the Physical Properties of Margarine Fats: Solid Fat Content, Crystallization Behavior, Crystal Morphology, and Crystal Network

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In this study enzymatic-interesterified margarine fats with different conversion degrees were produced in a packed-bed reactor. The effects of conversion degree on the formation of free fatty acids and diacyglycerols, solid fat content, crystallization behavior, microstructure, and crystal network were investigated, and the enzymatically interesterified products were compared with a chemically interesterified product. Formation of free fatty acids and diacyglycerols increased slightly with increasing conversion degree. The solid fat content was higher at 10 and 20 °C and lower at 30, 35, and 40 °C with increasing conversion degree. Increased conversion degree from the blend to products, measured by X-ray with addition of 50% of rapeseed oil for dilution, caused the content of β to decrease from 100% to 33%, and 30% and eventually to pure β' crystal. However, double chain packing was observed for both the blend and products. Isothermal crystallization kinetics was characterized by the Fisher−Turnbull model. The highest free energy was observed for the blend. A small deformation with oscillation tests shows a significant difference between the blend and interesterified products. The differences of microstructure between the blend, different conversion degree, and chemical randomized product were observed.

KEYWORDS: Lipozyme TL IM; interesterification; packed-bed reactor; crystallization; nuclear magnetic resonance (NMR); DSC, X-ray; polar light microscopy.

INTRODUCTION

Production of margarine fats is normally carried out by partial hydrogenation or interesterification. In the U.S., partial hydrogenation is mostly used for margarine fat production and has some drawbacks because of the formation of a high content of trans fatty acids during the process (*1*). There is great interest in producing trans-free products by interesterification of liquid oil with fully hydrogenated fats. In Europe, margarine hardstocks are normally produced by interesterified palm stearin and palm kernel or coconut oil. The specific choice of oils and fats depends on economics and availability (*2*).

Interesterification is performed to exchange fatty acids on the glycerol backbone. It can be carried out either chemically or enzymatically. The advantages of enzymatic interesterification are mild conditions and nutritional improvement or maintenance in the product. Also, there is easier control of the conversion degree for producing optimal products with the desired physical properties (*3*−*5*). The purpose of interesterification is not only to obtain satisfactory melting properties but also to obtain suitable crystallization behavior (e.g., more β' crystals) in order to produce higher quality margarine with no oiling off or sandy mouth feel.

Chemical interesterification usually leads to full randomization. It is difficult to terminate at a partial interesterification, since the reaction is very fast. Enzymatic interesterification is slower and can be more easily controlled. The conversion degree is critical for industrial applications because it is related to the reaction's productivity. Therefore, it is very important to know the effects of conversion degree on the physical properties of enzymatically interesterified products.

Most previous studies were based on fully converted products (*4*, *5*). In this study, a blend of palm stearin and coconut (70/30, w/w) was used. Lipozyme TL IM catalyzed interesterification was carried out in a packed-bed column. The effects of conversion degree on the formation of byproducts during the enzymatic process and on the physical properties of the products (such as solid fat content, crystallization behavior, microstructure, and crystal network) were studied. The comparison between chemically randomized and enzymatically interesterified products was also investigated.

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Table 1. Fatty Acid Composition of Lipids Used for study [wt %]

	palm stearin	coconut oil	rapeseed oil
C8:0	0	6.9	0
C10:0	0	6.0	0
C12:0	0.2	47.6	0.0
C14:0	1.2	18.3	0.0
C16:0	59.5	9.3	4.5
C18:0	5.0	2.9	1.7
C18:1	27.7	7.1	62.0
C18:2	5.8	1.9	19.4
C18:3	0.2	0.1	10.2
C20:0	0.4	0.0	0.6
C20:1	0.1	0.0	1.5

MATERIALS AND METHODS

Materials. Three different bleached, deodorized lipids (i.e., palm stearin, coconut oil, and low erucic acid rapeseed oil) were supplied by Karlshamns AB (Karlshamn, Sweden). The fatty acid compositions were analyzed by gas chromatography (**Table 1**). A blend of palm stearin and coconut oil in a weight ratio of 70/30 was used for interesterification study. Lipozyme TL IM, a silica-granulated *Thermomyces lanuginosus* lipase from Novozymes A/S, is a commercially available lipase and is used for interesterification reaction in a solvent-free system. All other chemicals and reagents for analysis were of analytical or chromatographic grades.

Chemical Interesterification. The blend (700 g) was dried under vacuum (less than 10 mbar) at 90 °C for 30 min. Afterward sodium methoxide (0.2%) was added to the reactor. The reaction was completed after about 30 min under vacuum. It was stopped by adding water at 70−80 °C. The product was washed five times followed by drying for 50 min under vacuum.

Enzymatic Interesterification in a Batch Reactor. Batch reactions (100 g batches) with two replicates were carried out in two 150 mL conical flasks with screw caps in a shaking water bath at 70 °C. Lipozyme TL IM (4%) was added afterward. The 50 μL samples were withdrawn from the reactor directly into 1 mL HPLC glass vials at times of 20 and 40 min and 1, 2, 3, 4, 6, and 24 h.

Enzymatic Interesterification in a Packed-Bed Reactor. A glass column (internal diameter = 1 cm, length = 24 cm) was used with a water jacket. An amount of 3 g of Lipozyme TL IM was wetted by rapeseed oil in a flask, and the air was removed under vacuum for 5 min. The enzyme slurry was transferred into the column that had 1−2 cm of glass beads at the bottom. After the enzyme slurry had settled down, the rest of the space was filled with glass beads. The whole system was held at 70 °C. The enzyme was equilibrated by running the blend through the column. The flow rate was kept at the lowest designed conversion degree for an hour to remove extra water in the enzyme in order to reduce the content of byproducts. Experiments were run consecutively with adjusting flow rates. Samples were collected after adjusting the flow rate and running the blend through the enzyme bed for at least three bed void volumes.

Conversion Degree of Enzyme-Catalyzed Interesterification. During enzymatic interesterification, the conversion degree can be controlled by using different reaction times. The conversion degree was defined as

$$X\,(\%) = \frac{P_t - P_0}{P_\infty - P_0} \times 100 \tag{1}$$

where P_t is the peak ratio at time t, P_∞ is the peak ratio at equilibrium, and P_0 is the peak ratio at time 0. During the reaction, the peaks ECN 44 and ECN 48 changed the most (**Figure 1**). Therefore, the peak ratio of ECN 44/ECN 48 was used for monitoring the process.

Relationship between Batch Reactor and Packed-Bed Reactor. To design a packed-bed reactor, the relationship between the batch and packed-bed reactor has to be determined. For a packed-bed reactor (*2*),

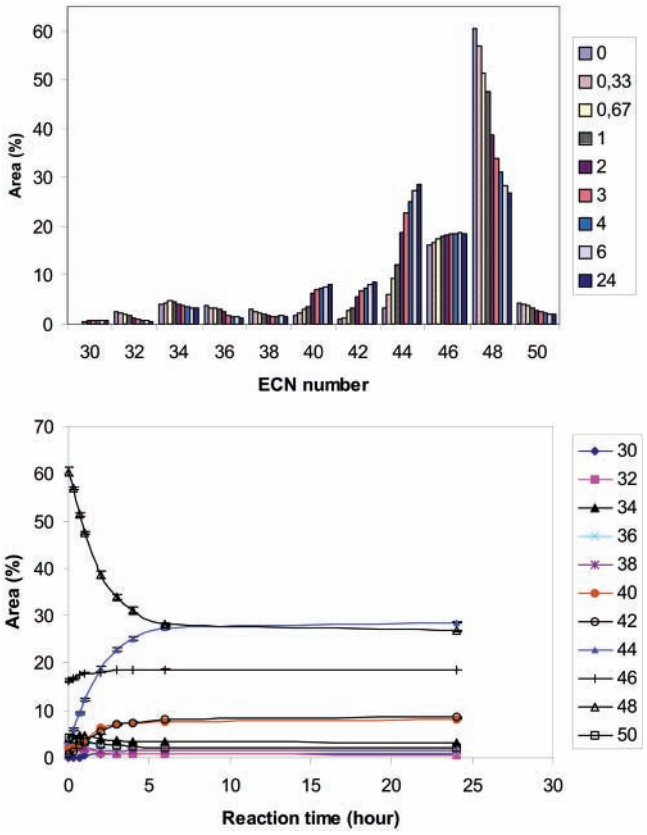

Figure 1. Evolution of the relative proportions of TAG as function of TAG equivalent carbon number (ECN) at different reaction times for lipase-catalyzed interesterification.

the relationship between enzyme load, flow rate, reaction rate, and conversion degree is as in

$$\frac{w_p}{F_p} = \int_0^{X_a} \frac{dX_a}{-r_a} \tag{2}$$

where w_p is the amount of enzyme in the packed-bed reactor, F_p is the flow rate through a packed-bed reactor, X_a is the substance conversion degree, and r_a is the reaction rate.

For a batch reactor (*2*), a similar relationship exists, which can be expressed as

$$\frac{w_b}{V_b} t = \int_0^{X_a} \frac{dX_a}{-r_a} \tag{3}$$

where w_b is the enzyme dosage, V_b is the amount of oil, and t is the reaction time.

Assuming the reaction rate and conversion degree are the same because the same enzyme is used, the right sides of eqs 2 and 3 are the same. Therefore, the following equation can be obtained:

$$\frac{w_p}{F_p} = \frac{w_b}{V_b} t \tag{4}$$

If no deactivation occurs in the packed-bed reactor, eq 4 can be written as follows:

$$F_p = w_p \frac{V_b}{w_b t} \tag{5}$$

On the basis of data experiments in the batch reactor, eq 4 can be used to predict the relations between the amount of enzyme and the flow rate in the packed-bed reactor. Products with the desired conversion degree can be obtained by adjusting the flow rate at a fixed enzyme load.

Differential Scanning Calorimetry (DSC). A Mettler DSC 822 was used. An amount of 8−12 mg of sample was sealed in a 40 μL aluminum pan. The transformation was studied and combined with analysis of X-ray results to identify the crystal form.

1. Thermal Dynamic Condition. Samples were heated from 20 to 90 °C at a heating rate of 20 °C/min and held at 90 °C for 5 min in order to totally melt them. They were then cooled at a rate of 5 °C/min to −60 °C and held for 10 min before heating to 90 °C, again at 5 °C/min. Onset and offset temperatures, major peak maximum temperatures (°C) during the exothermal and endothermal processes, and crystallization behavior were measured.

2. Isothermal Condition. Samples were heated from 20 to 90 °C at a heating rate of 20 °C/min and held at 90 °C for 5 min. Samples were cooled to the measurement temperature at 20 °C/min and held at this temperature for 1 h to determine the induction time. Samples were heated to 90 °C at a heating rate of 5 °C/min. Thermograms were analyzed for induction time and enthalpy of melting (J/g).

Thermodynamically, a supercooled melt is in a labile state. The molecules are in chaotic movement (*6*). An energy barrier to nucleation exists; unless local energy fluctuations are sufficiently large to overcome this barrier, nucleation will not occur and the supercooled system will remain in its metastable stage. This energy, which is the activation free energy of nucleation ΔG_c, can be evaluated by using the Fisher−Turnbull equation:

$$J = \left(\frac{NkT}{h}\right)\left[\exp\left(-\frac{\Delta G_d}{kT}\right)\right]\left[\exp\left(-\frac{\Delta G_c}{kT}\right)\right] \quad (6)$$

where J is the rate of nucleation, N is the number of molecules per cm^3 in the liquid phase, k is Boltzmann's constant, h is Planck's constant, T is the absolute temperature, ΔG_d is the free energy of diffusion, and ΔG_c is the free energy of nucleation.

A convenient way of determining J experimentally is to measure the induction time (t) of nucleation, which is inversely proportional to J. For a spherical nucleus, the activation free energy of nucleation is related to the surface energy of the crystal/melt interface σ and supercooling $\Delta T = T_m - T$ by

$$\Delta G_c = \frac{(16/3)\pi\sigma^3 T_m^2}{\Delta H^2 \Delta T^2} \quad (7)$$

where T_m is the melting point. The Fisher−Turnbull eq 7, which is originally derived for a single-component system, can also be applied to a multicomponent system. Plots of $\ln(tT)$ vs $1/T(\Delta T)^2$ are used, and the corresponding activation free energy of nucleation can then be evaluated from the slope s (*8*),

$$\Delta G_c = \frac{sk}{(T_m - T)^2} \quad (8)$$

where T is the temperature of crystallization.

Fatty Acid Composition. Analysis was by GC. Fatty acid methyl esters (FAMEs) were made by methylation with sodium methoxide and analyzed with a Famewax 0.25 mm × 30 m capillary column (Restek, Bellefonte, PA) in a Varian 3800 GC (Palo Alto, CA). Injection and detector temperatures were 220 °C. The initial oven temperature was 90 °C with a heating rate of 7 °C/min to 220 °C and a hold at 220 °C for 35 min. The carrier gas was helium at a flow rate of 3 mL/min.

Triglyceride Profiles. Triglycerides (TAG) were analyzed by reversed-phase HPLC. Separation was performed on a LiChroCART 250-4 (RP C18 end-capped) column (particle size of 5 μm, Merck, 64271 Darmstadt, Germany) with a binary solvent system of acetonitrile (solvent A) and dichloromethane (solvent B). Solvent B was increased from 25 to 39% over 15 min and kept at 39% for 10 min, then increased to 65% over 15 min, followed by 100% solvent B for 5 min, and then followed by the initial solvent A for equilibration of the system. An amount of 50 μL of sample was dissolved in 950 μL of chloroform, and 8 μL aliquots were injected. The peak identification was done by using standard TAGs with known equivalent carbon number (ECN). These were tricaprin, trilaurin, trimyristin, tripalmitin, and tristearin. The calculation of ECN value was described previously (*3*).

Diacylglycerol Content (DAG). They were analyzed on an HP narrow-bore silica column (l = 10 cm, i.d. = 2.1 mm, particle size = 5 μm; Hewlett-Packard, Waldbronn, Germany). A binary solvent system of heptane and heptane/tetrahydrofurane/acetic acid (80/20/1, v/v/v) was used. Samples were dissolved in heptane (5 mg/mL), and 20 μL aliquots were injected. Dipalmitin was used as the external standard, and calibration curves were established to quantify the amount of diacylglycerols in the samples. The concentration of diacylglycerol was expressed as the weight percentage of the sample.

Solid Fat Content (SFC). It was obtained by a Minispec mq 20 NMR analyzer (Bruker, Germany) according to the AOCS direct parallel measurement method (*9*).

Free Fatty Acid Content (FFA). This was determined by AOCS official methods (*10*).

X-ray Diffraction. A Siemens diffraction meter D5000 was used. Approximately 60 mg of fat was melted at 70 °C on a plastic plate and tempered at −18 °C for 15 min. X-ray measurement was at room temperature by programmed 7 times scanning determinations within 16 min for one sample. Polymorphic forms were determined from d spacings. The short spacing of the α form is at 4.15 Å. The β' form is at 4.2 and 3.8 Å. The β form has a very strong peak at 4.6 Å. The β-form crystal percentage compared with β' was calculated by the height of the peak (*11*), which is

$$\beta\text{-form crystal (\%)} = \frac{(\text{height of } \beta - \text{crystal at 4.6Å})}{(\text{height of } \beta - \text{crystal at 4.6 Å}) + (\text{height of } \beta' - \text{crystal at 4.2 Å})}$$

In triglycerides, there are normally two possible types of long spacing packing: double and triple chain packing with the length of two and three fatty acids, respectively. For fats containing fatty acids with 16 and 18 carbons in their chains, the lengths of double and triple spacings are about 45 and 65 Å, respectively.

Polar Light Microscopy. One drop of melted fat from a preheated glass capillary tube was put on a glass slide. A glass cover slip was carefully placed over the sample to give a homogeneous distribution. The sample was held at 90 °C for 5 min to melt the crystals. Then it was cooled to 28 °C within 2 min and kept at 28 °C for 1 h and monitored.

Samples with 50% added rapeseed oil were melted at 70 °C, and one drop was put on the slide. They were cooled to room temperature. The crystal morphology was monitored after 1 h at room temperature.

Rheological Measurement. Small deformations of the crystal network were carried out by concentric cylinder deformation rheometry (USD 200, Paar Physica, Germany) within the linear viscoelastic region. Plate−plate geometry with sanded surfaces was used. The sample was first held at 90 °C for 5 min to melt any crystals. It was cooled to 30 °C at 20 °C/min and equilibrated for 15 min before measurement. Oscillatory strain sweep over the range of 1×10^{-6} to 100% was carried out at a constant frequency of 1 Hz.

Large deformation of crystal network was by a TA-XT2 texture analyzer. Samples in glass beakers (diameter, 37 mm; height, 20 mm) were totally melted at 70 °C, and the beakers were left in a 5 °C refrigerator overnight. The measurements were carried out by using a 30° cone at a penetration depth of 7 mm and a penetration speed of 2 mm/s. Triple determinations were done at room temperature. The variation of force with measurement time was recorded. The maximum recorded force was used as the fat hardness.

Statistical Analysis. Data were statistically analyzed by SAS JMP 4.0 (Cary, NC).

RESULTS AND DISCUSSION

On the basis of the data from the batch reaction, the flow rate in the packed-bed reactor was calculated by using eq 5 with a lipase dosage of 3 g. Four products were produced at flow rates of 0.6, 0.4, 0.3, and 0.1 (g/min)/(g of lipase) at a temperature of 70 °C, and the conversion degrees were 31, 58,

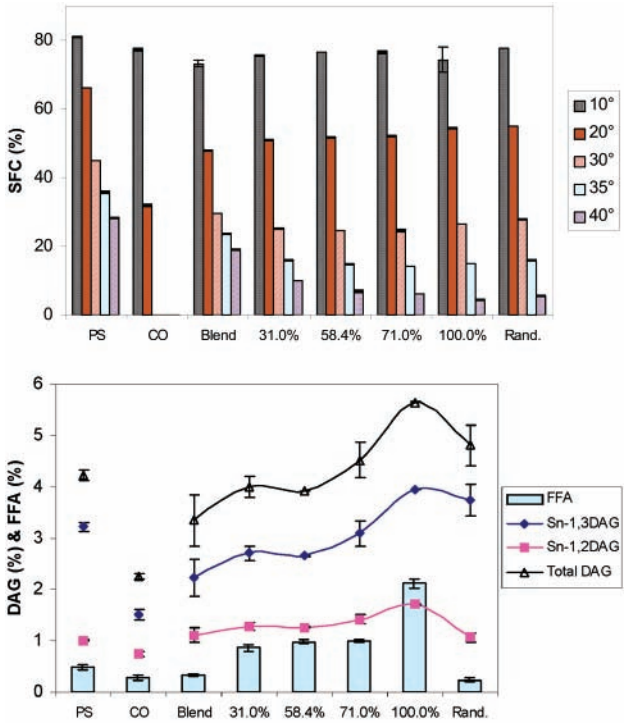

Figure 2. Contents of SFC at 10, 20, 30, 35, and 40 °C, DAG, and FFA for the feedstock, blend, and interesterified products. Abbreviations are the following: PS, palm stearin; CO, coconut oil; Rand., chemical randomized product.

71, and 100%, respectively. The chemically randomized product was also produced. **Figure 1** shows that during the enzymatic batch reaction, the ECN 32, 34, 36, 38, 48, and 50 decreased from 2.6, 4.0, 3.7, 2.9, 60.4, and 4.2% to 0.6, 3.2, 1.3, 1.5, 26.8, and 2.0%, respectively. However, the ECN 40, 42, 44, and 46 increased from 1.7, 0.9, 3.3 and 16.3% to 8.2, 8.5, 28.6, and 18.6%, respectively. This showed that fatty acid compositions on the glycerol backbone were changed during the reaction. New TAGs were formed during the interesterification by exchanging the fatty acids at TAGs' backbone. The content of TAG from coconut with only shorter chain length (ECN32, 34, 36, 38, 40) was decreased (except ECN40). Palm stearin has a longer chain length (ECN46, 48, 50). These were decreased after interesterification (except ECN46). The peak areas of ECN 40, 42, and 44 were increased after interesterification as the molecular composition of the samples became more uniform. The exception to this pattern was ECN 46, which changed little during the reaction. Rousseau et al. (*12*) observed the same for lipozyme RM IM catalyzed interesterification in butter fat modification. Because of the changes of chemical composition during interesterification, related physical properties were also changed. **Figure 2** shows SFC, DAG, and FFA for the original feedstocks, the blend, and the interesterified products. It can be seen that SFC was slightly increased at 10 and 20 °C and significantly decreased at 30, 35, and 40 °C after interesterification (**Figure 2**, top). The blend of palm stearin and coconut oil at a ratio of 70/30 had a slight interaction for both enzymatically and chemically randomized products. That might be due to coconut oil, which has a high content of saturated lauric and myristic acids and shows very sharp melting. After interesterification, the fatty acid chains (mainly palmitic) at the sn-1,3 position were exchanged and the liquid TAG at lower temperature in the blend became more solid-like as long saturated fatty acids bonded with the glycerol backbone. Longer-chain-length TAGs decreased in overall size because of the interesterification, and

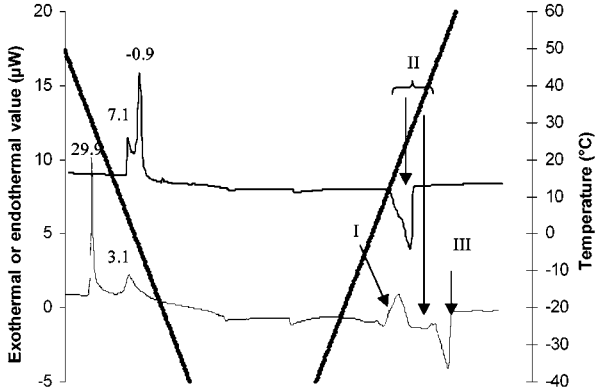

Figure 3. DSC profiles of palm stearin and coconut oil.

so at higher temperatures there is a decrease in solids content. Chu et al. (*4*), using palm stearin and palm kernel olein, did not find this phenomenon for the product with a blend ratio of 1:1 catalyzed by *Rhizomucor miehei* lipase at 60 °C for 6 h. The interesterified products showed lower SFC compared to the blend at all temperatures studied.

FFA contents were slightly increased with higher conversion degree due to the longer reaction time (**Figure 2**, bottom). DAG showed the same tendency as FFA. In agreement with our previous study, FFA slightly increased with increased reaction time and the content of DAG followed the changes of FFA in the system (*13*). However, if the enzymatic process was well controlled with equilibrated enzyme and controlled water content from feedstocks by the drying process, byproducts can be significantly decreased. This enables manufacturers to obtain the demands of product quality directly after enzymatic interesterification.

Both fatty acid and triglyceride compositions contribute to the melting and crystallization properties of the fat blends (*8*, *14*). **Figure 3** shows the DSC profiles of palm stearin and coconut oil. From the exothermal peaks of palm stearin, it can be seen that there are two very separate peaks, peak 1 at 29.9 °C belonging to TAG composed of highly saturated fatty acids; the second peak at 3.1 °C was mainly of TAG composed of unsaturated fatty acids. The broadening effect between the two peaks was due to the wide TAG compositions in the palm stearin. It was observed by Ng that the broadening effect could be limited by removal of the "hard" components from the oil (*8*). However, coconut oil showed two very close exothermal peaks. Because the fatty acid composition of coconut oil is mainly medium-chain-length saturated fatty acids, which have similar chain lengths and have a very small amount of unsaturated fatty acids (**Table 1**), it is relatively homogeneous and so the physical properties of the TAGs are quite similar. Thus, no broadening effect was found. On heating, palm stearin showed crystal transformation through three crystal forms (here, designated I, II, and III), and then it was totally melted at 56.4 °C. Coconut oil showed only crystal form II (β' crystal), with a slight shoulder caused by the differing TAG composition.

Lipozyme TL IM is an sn-1,3 specific lipase. Fatty acids were exchanged during the reaction only at the sn-1,3 position. The fatty acids at the sn-2 position remain unchanged except that acyl migration took place with a higher content of byproducts in the system (*15*). However, chemical randomization totally rearranged the fatty acid composition in the glycerol backbone. The broadening effect due to the differences of TAG composition in the blend became smaller after chemical randomization than enzymatic interesterification. This can be seen in **Table 2** or in the DSC profiles in **Figure 4**. After interesterification,

Table 2. Onset and Offset Temperatures from Dynamic DSC Measurements and Exothermal Peaks Temperatures for the Feedstock, Blend, and Interesterified Products

content	exothermal (°C) onset	exothermal (°C) peaks	endothermal (°C) offset
palm stearin	31.31 ± 0.13	29.9, 3.1	56.43 ± 1.09
coconut	8.03 ± 0.10	7.1, −0.9	26.12 ± 0.43
blend	27.56 ± 0.15	26.9, 1.9, −5.6	53.87 ± 0.01
31% conversion degree	25.11 ± 0.03	24.7, 5.7, 0.3	44.74 ± 0.12
58.4% conversion degree	24.51 ± 0.05	24.0, 6.7, 1.2	41.50 ± 1.85
71% conversion degree	24.25 ± 0.04	23.9, 7.1, 1.4	43.20 ± 0.10
100% conversion degree	24.14 ± 0.08	23.7, 8.1	42.79 ± 0.08
chemical randomized product	24.26 ± 0.33	23.5, 13.3	43.09 ± 0.05

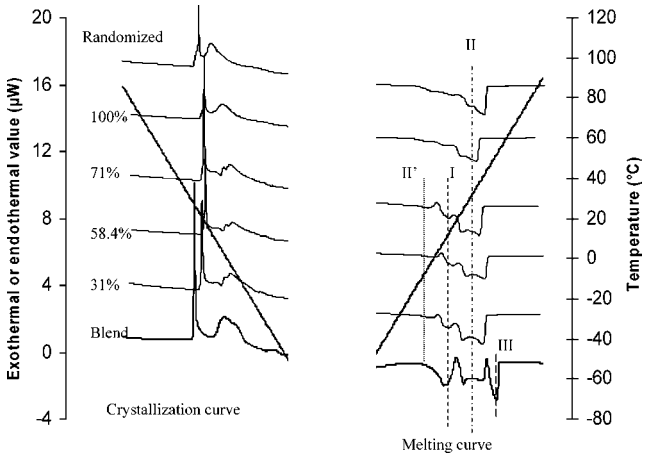

Figure 4. DSC profiles of the blend and its interesterified products.

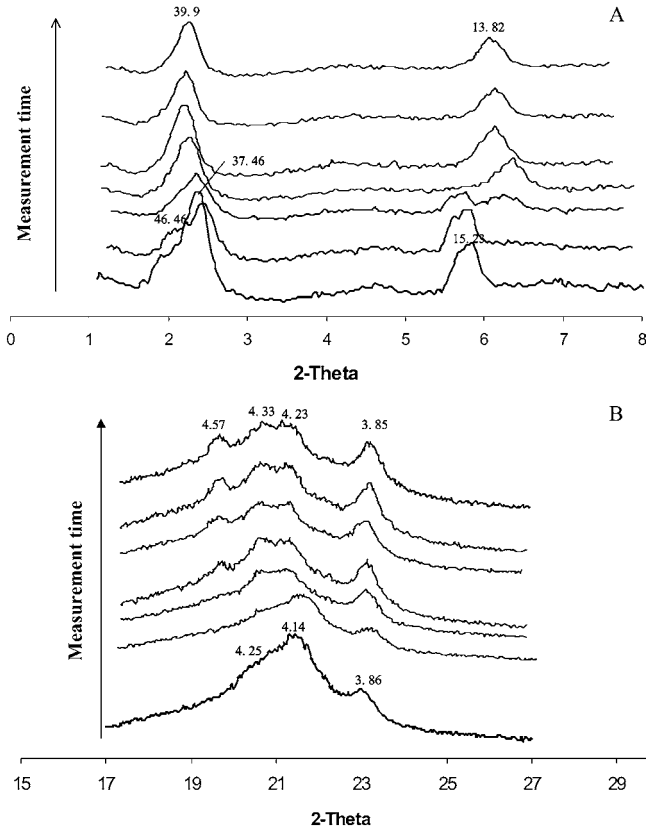

Figure 5. X-ray profiles of long and short spacing based on the blend at room temperature by programmed 7 times scanning determination in 16 min.

crystallization behavior was altered because of a change of composition. Overall, exothermic peaks were still mainly divided into two parts. However, with increasing conversion degree, the broadening effects became smaller than for the original blends because of increasingly homogenized TAG distribution of the different chain lengths (**Table 2**). The second peak with a slight shoulder at the beginning from 1.9 and −5.6 °C became one peak at 8.1 °C. The temperature difference of the two peaks decreased from 25.0 to 10.2 °C. The smallest broadening effect was observed for the chemically randomized product. This indicates that the differences in the chemical compositions of TAG affect physical properties. The onset temperature of enzymatically interesterified products was significantly decreased from 27.6 to 24.1 °C ($P < 0.05$), and the offset temperature was decreased from 53.9 to 42.8 °C (**Table 2**), which was mainly because of the decrease of tripalmitin after interesterification. From the endothermic profiles, it can be seen that the crystal behavior was changed after interesterification. At a heating rate of 5 °C/min, no crystal form III was found for any interesterified products, including enzymatically and chemically randomized products. The crystal forms of enzymatically interesterified products at conversion degrees of 31, 58.4, and 71% have three different types of crystal that we designate II′, I, and II. However, the fully interesterified product and chemical randomized product show mainly crystal form II.

X-ray analysis (**Figure 5**) was used to characterize the TAG polymorphic form and the chain packing of fatty acid end groups, which corresponded to a single or multiple chain lengths of TAG. The common type of solid-state structure for saturated glycerides is a double structure with reversed stacked turning forks (*16*). Unsymmetrical glycerides may give triple-chain-length structures. It is clearly seen that the blend of palm stearin (46.46 Å) and coconut oil (37.46 Å) was double packed (**Figure 5A**). There was the same double packing for the enzymatic interesterified and chemical randomized products (unpublished data). No effect on the TAG packing after interesterification was found. With increasing scanning time, the temperature increased to room temperature. The peak at 37.46 Å disappeared, and the peak at 46.46 Å moved toward 39.9 Å. That was caused by the tilting of the TAG chain from the very unstable crystal α to β' and finally to stable β. At the same time, it is observed that the trisaturated fatty acids in the mixture have dominant effects on the crystal transformation. Long spacing for α, β', and β are 45.6, 42.3 and 40.6 Å (16). This agreed with our observation. The coconut oil was partially melted and had lower solids content at room temperature. Therefore, it did not show significant effects on the crystal behavior in the blend. Polymorphic transformation can be clearly seen from the short spacing (**Figure 5B**). At the first scan, the crystals were mainly in the α form, with a little β' from coconut oil. With an increase of time and temperature, there was only β' at the third scan after 6 min at 22 °C. However, it soon transformed into a blend of β' and β. β increased with time and temperature. This was

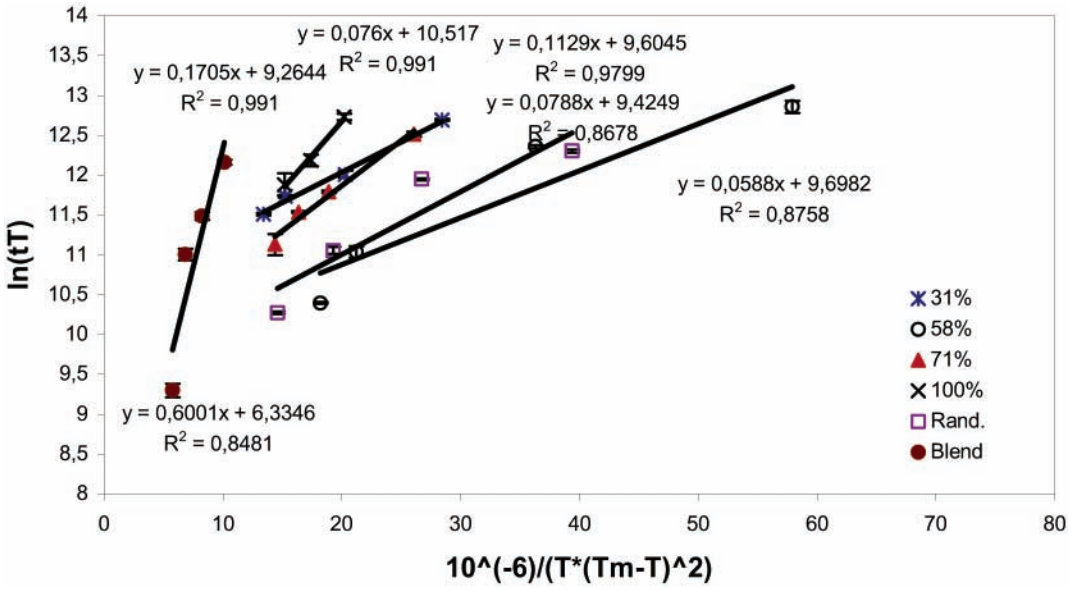

Figure 6. Fitting of the nucleation kinetics of the blend and interesterified products to the Fisher–Turnbull equation. For definitions of the abbreviations, see the caption of **Figure 2**.

Table 3. Linear Relationship between the Plots of ln(tT) vs $1/T(\Delta T)^2$

	linear equation	r^2
blend	$y = 0.6001x + 6.3346$	0.84
31% conversion degree	$y = 0.076x + 10.517$	0.99
58% conversion degree	$y = 0.0588x + 9.6982$	0.87
71% conversion degree	$y = 0.1129x + 9.6045$	0.98
100% conversion degree	$y = 0.1705x + 9.2644$	0.99
chemical randomized product	$y = 0.0788x + 9.4249$	0.87

confirmed by the DSC results (**Figure 4**) showing that crystal I is α and that crystal II is β'. The blend was further equilibrated at 45 °C for 15 min. The crystal form was examined by X-ray analysis. By now, it was totally transformed to β (data not shown here). That was further confirmation that crystal form III is β. II′ was not detected during the tests. It might be due to the quick transformation affected by the changing of temperature from −18 °C to room temperature. Crystal form II′ might be γ (sub-α) or called β'_2.

According to the Fisher−Turnbull eq 8, the free energies were calculated on the basis of the plots of ln(tT) vs $1/T(\Delta T)^2$ (**Figure 6**). s values were obtained from the slope (**Table 3**). It can be clearly seen that after interesterification, the slope was significantly decreased. There is a tendency of slope decrease with increase of interesterification degree. The calculated free energy was shown in **Table 4** over the temperature range from 301 to 309 K. The blend has a consistently higher activation free energy ΔG of nucleation than interesterified products. As the temperature increased, crystallization became more difficult because of the lower driving force for nucleation since the blend was more β tending than the interesterified products. The crystallization of β needs more energy than β' crystallization. Also, more energy is needed to melt the blend's crystals because the β in the blend is much more stable than the β' in the interesterified products. Toro-Vazquez et al. (*17*) studied the blend of palm stearin with sesame oil in different ratios. They found that despite the higher concentration of palm stearin,

Table 4. Free Energies at the Isothermal Crystallization Conditions and Enthalpy Energies at a Heating Rate of 5 °C/min after 1 h of Crystallization for the Blend and Interesterified Products[a]

				conversion degree (%)					
	blend			31			58		
temp (K)	ΔG (kJ/mol)	ΔT (K)	enthalpy (J/g)	ΔG (kJ/mol)	ΔT (K)	enthalpy (J/g)	ΔG (kJ/mol)	ΔT (K)	enthalpy (J/g)
301							2.7	13.5	−27.5
302				2.6	15.7	−27.9	3.1	12.5	−26.1
303	8.7	23.9	−34.2	3.0	14.7	−24.1			
305	10.4	21.9	−29.8	4.0	12.7	−22.4	5.4	9.5	−20.9
307	12.6	19.9	−27.2	5.6	10.7	−17.5	8.7	7.5	−10.3
309	15.6	17.9	−25.5						

	conversion degree (%)								
	71			100			chemical randomized product		
temp (K)	ΔG (kJ/mol)	ΔT (K)	enthalpy (J/g)	ΔG (kJ/mol)	ΔT (K)	enthalpy (J/g)	ΔG (kJ/mol)	ΔT (K)	enthalpy (J/g)
301	4.1	15.2	−27.5	6.5	14.8	−27.8	2.9	15.1	−28.8
302	4.6	14.2	−25.9	7.4	13.8	−26.0			
303	5.4	13.2	−24.4	8.7	12.8	−24.6	3.8	13.1	−23.0
305	7.5	11.2	−20.4				5.3	11.1	−18.4
307							7.9	9.1	−16.8
309									

[a] ΔG is the free energy. $\Delta T = T_m - T$.

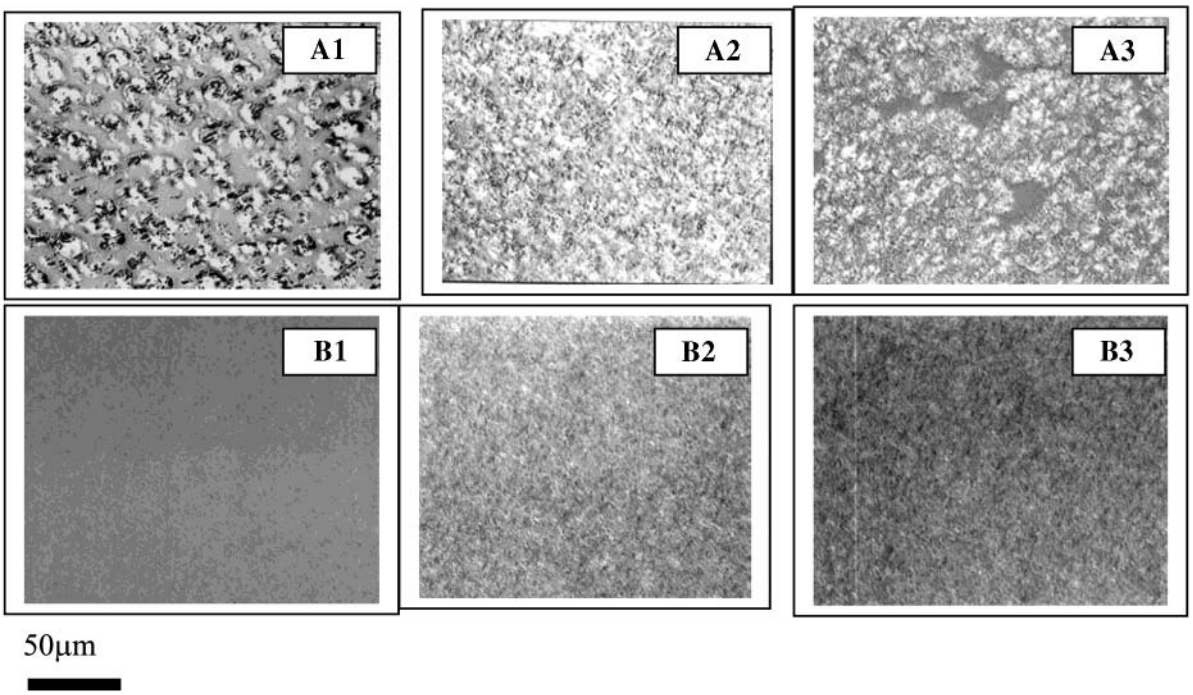

Figure 7. Morphology of the blend and enzymatic fully interesterified products after fast cooling from 90 to 28 °C at the observation times of 0, 5 min, and 1 h.

which was 80% palm stearin blended with sesame seed oil, crystallization in the β crystal form required more free energy for its development than β' crystallization in the blend ratios of 26, 42, and 60% palm stearin with sesame oil.

The crystal morphologies of the blend and its interesterified products were observed on a hot-stage polar light microscopy. A mixture of dotted and feather-like crystals were observed for the blend and enzymatically interesterified products with the conversion degree lower than 71% at 28 °C (data not shown here). The dotted spherulites were related to the α crystal form. Kawamura (*18*) observed for palm oil that pure dotted α spherulites were found at the beginning of isothermal crystallization, when the temperature was below 24 °C. However, two types of crystals existed in this study. This suggests that α and β' forms coexisted. This was confirmed by X-ray results. Enzymatically interesterified products at full conversion (part **B1** of **Figure 7**) and chemically randomized products (data not shown here) showed needle-like crystals forming. These were very well correlated with DSC profiles (**Figure 4**) that showed that the blend and interesterified products were α (except for fully converted enzymatically interesterified product and the chemically randomized product). Crystals grew simply by surface nucleation and molecular transportation by diffusion. After 5 min, secondary nucleation began. Crystal groups formed as new crystals nucleated next to existing crystals. After 1 h, the blend showed very condensed clusters that had a clearly separated phase from the liquid oil (part **A3** of **Figure 7**). However, the interesterified products had a very homogeneous network that could hold the liquid oil inside the network without any separated phase even with lower SFC after interesterification (part **B3** of **Figure 7)**. Because of the high content of solid fat in both blend and products, it was difficult to see a clear relationship between of crystal shape and conversion degree, and so 50% of rapeseed oil was added to dilute the system. The photographs taken after cooling in room temperature for 1 h are shown in **Figure 8**. It is clearly shown that the blend formed very coarse, condensed, branched, and leaflike crystals. The size was bigger than 50 μm. Interesterified products showed significantly different morphologies compared with the blend. The appearance of crystals was from coarse to fine with increasing conversion degree. The product with 31% conversion degree had a mixture of leaf- (and more), ball-, and needle ball-like spherulites, and the longest dimensions were about 50 μm. It has been confirmed by X-ray crystallography that about 33% β existed in the products and that β' and β coexisted. Crystal sizes decreased and leaflike crystals disappeared with increasing of conversion degree. When the conversion degree was over 70%, only needle- or needle ball-like crystals were observed. This was the β' crystal form (confirmed by X-ray analysis). The fat crystal network provides firmness or solid-like behavior. Many of the sensory attributes such as spreadability, mouth feel, texture, etc. are dependent on the mechanical strength of the underlying fat crystal network (*19*). Rheological behavior is controlled by interaction between the fat crystals. It can be characterized by using small or large deformation tests. The large deformation tests usually lead to irreversible structure breakdown of the fat crystal network. Large deformation was performed by the texture analyzer. **Figure 9** shows that palm stearin was harder than coconut oil because of the longer chain length. The blend and interesterified products did not show significant differences ($P > 0.05$). Haighton (*20*) found that the hardness of margarine (a water in oil emulsion) had power law relationship with the solid content index. However, in this case there were no significant differences in hardness at 5 °C of the blend or interesterified products. The small deformation tests were carried out by oscillation. The relation between G', which is the storage modulus, and G'', which is the loss modulus, is represented by $\tan\delta = G''/G'$, in which $\tan\delta$ is a damping factor. Higher $\tan\delta$ values indicate less elastic and more viscous properties. **Figure 10** shows the oscillatory tests at 30 °C with controlled strain at a frequency of 1 Hz. The significant differences were found between the blend and the interesterified products for the ratio of G''/G'. It shows that the crystal network of the blend was more cohesive and had less elasticity. However, interesterified products are more elastic than the blend.

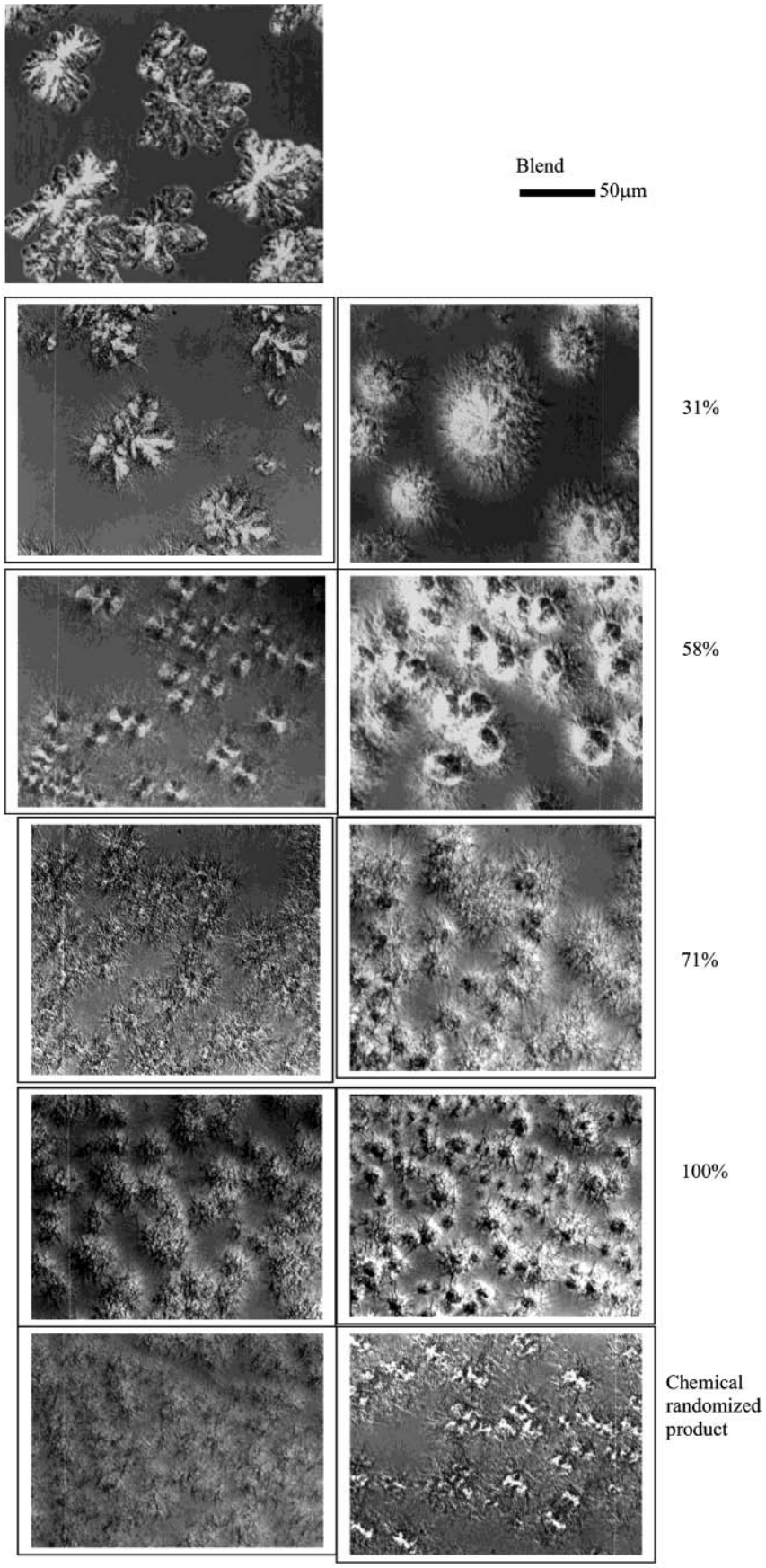

Figure 8. Morphology of the blend and interesterified products with addition of 50% of liquid rapeseed oil.

From this study, the interesterification degree does have significant influence on the physical properties because of the changes of chemical composition during the reaction. SFC curves became sharper, and enzymatic products are more β' tending with increasing degree of interesterification. Crystal morphology and X-ray diffraction show that two different kinds

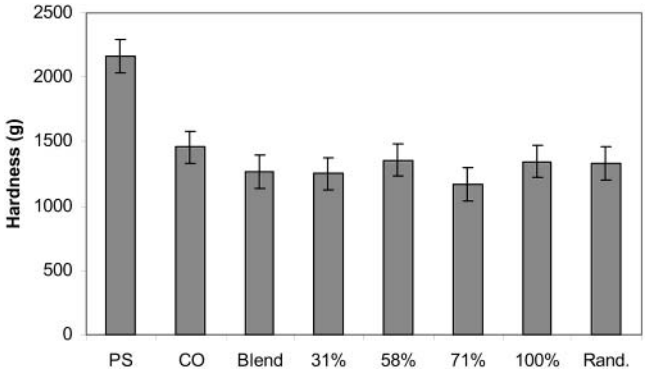

Figure 9. Hardness tests for the feedstocks, blend, and interesterified products. For definitions of the abbreviations, see the caption of **Figure 2**.

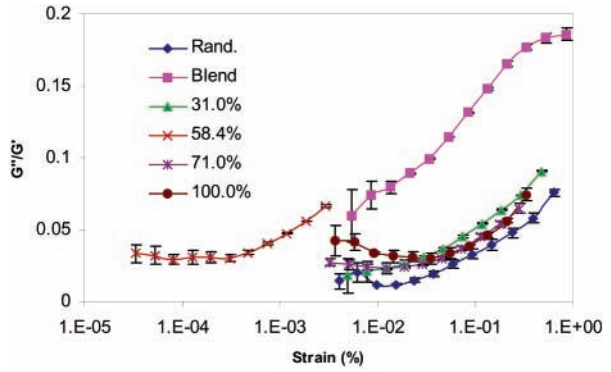

Figure 10. Oscillation tests at a frequency of 1 Hz and 30 °C for the blend and interesterified products. For definitions of the abbreviations, see the caption of **Figure 2**.

of crystals, such as α and β' or β' and β coexisted. Crystallization behavior was controlled by high-melting trisaturated tripalmitin. The crystal network was changed and showed more elastic properties for interesterified products by oscillatory tests. However, cone penetration did not show any significant differences. Overall, the blend of palm stearin and coconut oil either enzymatically interesterified or chemically randomized could be suitable for margarine hard stock production.

ABBREVIATIONS USED

GC, gas chromatography; FAMEs, fatty acid methyl ester; HPLC, high-performance liquid chromatography; ECN, equivalent carbon number; FFA, free fatty acids; DAG, diacylglycerides; TAG, triglycerides; SFC, solid fat content; DSC, differential scanning calorimetry.

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