Effect of Sucrose Esterified Fatty Acid Moieties on the Crystal Nanostructure and Physical Properties of Water-in-oil Palm-based Fat Blends

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Abstract: The effects of sucrose ester of fatty acid (SEF) on the nanostructure and the physical properties of water-in-oil (W/O)-type emulsified semisolid fats were investigated. Model emulsions including palm-based semisolid fats and fully hydrogenated rapeseed oils with 0.5% SEF or fractionated lecithin, were prepared by rapidly cooling crystallization using 0.5% monoacylglycerol as an emulsifier. The SEFs used in this study were functionalized with various fatty acids, namely, lauric, palmitic, stearic, oleic, and erucic acids. Cryogenic transmission electron microscopy (cryo–TEM) was used to observe the sizes of the solvent-extracted nanoplatelets. The solid fat content (SFC), oil migration value, and storage elastic modulus were also determined. The average crystal size, which was measured in length, of the fat blends with SEFs containing saturated fatty acids (namely, palmitic and stearic acids) was smaller than that of the SEFs containing unsaturated fatty acids (namely, oleic and erucic acids). The effects exerted by these fatty acid moieties on the spherulite size in the corresponding bulk fat blends were observed via polarized microscopy (PLM). The results suggest that nanostructure formation upon the addition of SEF ultimately influenced these aggregated microstructures. Generally, smaller platelets resulted in higher SFC in the fat phase, and a high correlation between the SFC and the G’ values in W/O emulsion fats was observed (R² = 0.884) at 30°C. In contrast, the correlation was low at 10°C. Furthermore, samples with larger nanocrystals had a higher propensity for oil migration. Thus, the addition of SEF regulated the fat crystal nanostructure during nucleation and crystal growth, which could ultimately influence the physical properties of commercially manufactured fat products such as margarine.

Key words: sucrose esters, nanostructure, fatty acid, physical properties, cryo–TEM

1 Introduction
Margarine and table spreads are fat products composed of multiphase colloidal systems containing dispersed water droplets in a continuous fat phase. These water-in-oil (W/O) emulsions mainly consist of semisolid fats, water, and emulsifiers. High-melting fats, such as palm oil and fully hydrogenated rapeseed oil, are used as semisolid fat ingredients in these mixtures to form three-dimensional crystal network structures. The physical properties of these fat products, relating to spreadability and hardness, are due to the above-mentioned crystals. Fat products are usually manufactured using rapid cooling and shearing processes, which, in turn, can influence the crystal behavior and subsequent mechanical properties of the products.

Over the dispersed water droplets generated during the manufacturing process can also influence the physical properties of these fat spreads by changing the crystallization traits of the fat content in W/O emulsions, which ultimately affects product quality. The physical properties of these fat products are influenced by the solid fat content (SFC), the size and shape of the fat crystals, the crystalline spatial arrangement, and the intercrystalline interactions due to van der Waals forces. The oil binding capacity of fat products is also a function of several complex factors. Thus, controlling the extent of crystallization in these fat products is important.

Amphiphilic molecules, such as sucrose ester of fatty acid, polyglycerol ester of fatty acid, and sorbitan ester of
fatty acid, are widely used in the food manufacturing industry to improve the properties and morphology of fat crystals\textsuperscript{14, 13}. Sucrose ester of fatty acid (SEF) is employed in a wide range of products because it is odorless and tasteless. SEF is also used in the pharmaceutical and cosmetic industries, where its nonionic and biodegradable properties are desired. Since SEF is a manufactured additive, the type and degree of esterification of the fatty acid moiety, and thus, the fat product’s hydrophobic character, can be adjusted to suit the intended use. Several studies have reported on the effects of fatty acid esterification on the fat crystallization behavior of SEF by using bulk phase and oil-in-water emulsified systems\textsuperscript{16–24}; however, very few studies have been conducted on the effects of the nanostructure on the physical properties of the fat product in W/O emulsion systems, which model fat spreads, or margarine.

Transmission electron microscopy (TEM) is an effective technique for observing nanoscale-sized fat crystals. Acevedo and Marangoni developed a practical method for quantifying the smallest fat crystal unit structure. This technique incorporated a nanoplatelet extraction method using cold isobutanol prior to cryo–TEM analysis, so that the effects exerted by the nanoplatelet structure on the physical properties of semisolid fats can be observed\textsuperscript{16, 11, 20}.

This study aimed to investigate the effect of nanostructure fat crystals caused by SEF on the physical properties of W/O emulsions, including the influence exerted on semisolid fats due to rapid cooling crystallization. We examined how the type of fatty acid moieties esterified to sucrose could affect the size of the nanoplatelets using SFC, oil migration, and the storage elastic modulus as the measured parameters. The addition of SEF was observed to influence aggregated microstructures, and the smaller platelets provided higher SFC in the fat phase, relating to higher elastic modulus values in W/O emulsion fat blends at 30°C.

The present study attempted to show that the relationship between the nanostructure and the physical properties of fat products is controlled by the nature of SEF. The results of this study will enable manufacturers to improve food formulations for the production of various kinds of fat spreads and margarine.

2 Experimental

2.1 Materials

Soybean oil, palm oil, and fully hydrogenated rapeseed oil were purchased from the Nisshin OilliO Group, Ltd. (Tokyo, Japan). The distilled monoacylglycerols, including typical saturated fatty acid-based monoacylglycerol (43.0% palmitic acid, 54.6% stearic acid, and 2.4% others), were obtained from Kao Co. (Tokyo, Japan). The fatty acids for the SEFs include stearic type 1 (S-170, HLB = 1), stearic type 2 (S-370, HLB = 3), oleic (O-170, HLB = 1), lauric (L-195, HLB = 1), palmitic (P-170, HLB = 1), and erucic (ER-190, HLB = 1) fatty acids, which were supplied by Mitsubishi Chemical Foods Co. (Tokyo, Japan). The ratio of monois-, di-, tri-, and polyesters of P-170, S-170, O-170, and L-195 was 1:99. While that of ER-190 was 0:100, and that of S-370 was 20:80\textsuperscript{16, 22}. Fractionated lecithin obtained from soybean oil was supplied by Riken Vitamin Co., Ltd. (Tokyo, Japan). The fatty acid composition of the lecithin was reported to be 14.8% (C16:0), 12.3% (C18:1), 62.3% (C18:2), and 6.9% (C18:3)\textsuperscript{20}. The effect of the different base structures of lecithin and SEF on the fat microstructure was determined to obtain comparative data.

The formulations of the model W/O-type emulsified samples consisted of 48.0% soybean oil, 20.0% palm oil, 2.0% fully hydrogenated rapeseed oil, 0.5% monoacylglycerol, and water. The water content was 29.0% and 29.5% in the samples with and without 0.5% SEF, respectively. The fatty acid compositions of the fat blends were 19.5% palmitic acids, 5.3% stearic acids, 26.7% oleic acid, 38.9% linoleic acid, 4.7% linolenic acid, and 1.6% behenic acid. Semisolid fat blends were examined to determine their nanostructure and physical properties. These included fat blends without SEF and lecithin (control), with L-195 (L–SE), P-170 (P–SE), S-170 (S–SE), O-170 (O–SE), ER-190 (ER–SE), S-370 (SH–SE), and with fractionated soybean lecithin (LE).

2.2 Preparation of the W/O emulsions and the bulk fat blends

Fat blends with added monoacylglycerol were melted and maintained at 80°C for 30 min to erase all crystal memory from the structure. Then, the water phase was added to the fat blend and mixed using a homogenizer (Ultra-Turrax T25 disperser, Ika Werke GmbH & Co. KG, Staufen, Germany) at 50°C. The pre-emulsified mixes (300 g) were added to a stainless container (8 cm diameter and 8 cm deep), equipped with a batch-type scraped surface chiller system in which the walls of the container in direct contact with the sample mixes were cooled to −20°C using a circulating liquid refrigerant. The mixture was placed in the container with 2-blade propellers rotating at 600 rpm and was rapidly cooled from 50 to 10°C in 300 s. The temperature of the mixture was monitored by a thermometer dredged through the mixture and the cooling temperature curve obtained was similar for all samples. Immediately after achieving 10°C, the crystallized W/O emulsions were placed in polypropylene containers and kept at 5°C until the relevant analysis had been conducted. The average size of the water droplets in the SEF was between 2.08 μm (minimum, SH–SE) and 3.02 μm (maximum, O–SE). The size of the LE droplets was 1.60 μm, which was relatively small when compared with the other samples.
2.3 XRD measurements

We conducted XRD measurements (Rint-Ultima 2000, CuKα: λ = 1.54 Å; Rigaku Corp., Tokyo, Japan) to determine the small-angle diffraction patterns (2θ = 0 – 6°) and to calculate the long spacing values of the crystal polymorphs. The XRD spectra profiles were calculated by subtracting the blank data. The W/O emulsion fat samples were maintained at 5°C during the measurement experiments. The crystallite size (d) was calculated by applying the Scherrer equation to the full width at half maximum (FWHM) of the XRD peak \[2θ \text{rad/s}\]. The d values appeared to agree with the nanoplatelet thickness and lamellar height taken from side view observations via cryo–TEM\(^1\).

The chain packing subcell structures, which are polymorphic forms of the relevant fat blends, were determined by wide-angle XRD. Quantitative estimations of the proclivity for the β-form were based on the ratio of the peak height of the β-form (0.46 nm) to the β’-form (0.42 nm); these values were obtained from the XRD peak intensity\(^2\), \(^3\).

2.4 SFC measurements

The SFC of the fat phases of the samples was determined with an NMR minispec mq20 (Bruker Optik GmbH, Ettlingen, Germany). Here, melted bulk fat blends (4 mL) were placed in NMR tubes (ID = 0.8 cm, L = 18 cm) and then immersed in a water bath at 80°C to erase all crystal memory from the sample. Next, these tubes were kept at 5°C for 30 min in the water bath, and the SFC value was measured. The sample tubes were then transferred to a water bath set at the crystallization temperature (10°C), and another round of SFC readings was obtained after 30 min. These steps were repeated at intervals of 5°C, for the temperature range from 5 to 35°C. All SFC measurements were done in triplicate. In this study, the SFC from the solid-state of SEP was presumed sufficiently small compared to that of fat (0.7% SEP). To elucidate the effect of surfactants of the SFC of saturated fatty acids solidified via co-crystallization with fat, further investigation using DSC is required.

The melting property of the SFC of the samples was confirmed to be adequate for measurements taken at 30 min intervals in contrast to crystallization by preliminary determination.

2.5 Dynamic rheological measurements

The storage modulus, \(G'\), obtained from constant frequency dynamic compression experiments, was selected as the rheological parameter to be investigated, as solid fat-like behavior is described well by the storage modulus. Dynamic experiments were performed between parallel plates using a rheometer (Rheometrics, TA Instruments, New Castle, DE, USA). The sample thickness and radius were 2 and 12.5 mm, respectively. A small frequency (1.0 rad/s) and strain (0.5%), which have linear viscoelastic behavior, were applied while the sample was heated from 5 to 30°C at the rate of 0.5°C/min. Representative profiles of W/O emulsion samples are shown in the figure, and their repeatability was confirmed.

2.6 Fatty acid analysis

The fatty acid composition of the fat blends and monoacetylglycerol were determined according to the AOCS Official Ce 1c-89 protocol\(^4\). GLC analysis was conducted using an HP5890 (Agilent Technologies, Inc., Palo Alto, CA, USA) equipped with an FID operating at 300°C. The SP-2560 column (Supelco, Inc., Bellefonte, PA, USA) was used as the capillary column and the injector port temperature was set at 250°C. The analyses were conducted by ramping the temperature from 180 to 200°C at a rate of 2°C/min after an initial holding time of 45 min at 180°C.

2.7 Observation of water droplets in emulsion

The average sizes of the water droplets in the samples were determined using a confocal laser microscope, FLUOVIEW FV1000 (Olympus Co., Tokyo, Japan), and an optical scanner GT-X820 (Seiko Epson Co., Suwa, Japan).

2.8 Pretreatment for the extraction of the nanoplatelets

The sample pretreatment procedure for the extraction of nanocrystals was conducted as previously reported\(^5\). Briefly, the nanocrystals were separated from the fat samples using a cold solvent-based extraction method, followed by cryo–TEM imaging. The fat blend samples (0.8 g) were suspended in cold isobutanol (10 mL). The mixture was then homogenized at 24,000 rpm for 10 min using a homogenizer attached to the disperser element S10N-10G (Ultra-Turrax T25 disperser, IKA Werke GmbH & Co. KG, Staufen, Germany). The mixture was filtered under vacuum through a membrane filter with a pore size of 1.0 μm, and the resulting crystals were collected using a spatula. The isolated crystals were suspended in a cold mixture of isobutanol (10 mL) and water (10 mL) before being homogenized for 10 min and then subjected to vacuum filtration again. The recovered crystals were suspended in cold isobutanol and were sonicated for 20 min using a Branson 3510J-MTH sonicator (Yamato Scientific Co., Ltd., Tokyo, Japan). During sonication, ice-cold water was used in the water bath to prevent a temperature increase. This sonication step helps to disperse nanoplatelets that may have become aggregated during the filtration steps. The temperature of the suspended samples remained under 10°C during this step.

2.9 Cryo–TEM observation

Approximately 4 μl of each sample mixture was placed on a carbon grid with a perforated carbon film (C-flat CF-2/2-4C, Protochips, Morrisville, NC, USA) at 10°C. The excess liquid was blotted off using a filter paper for 4 s. A
of the crystals against the solvent, after which the excess solution was again blotted off using a filter paper for 4 s. After a further 2 s, the grid was dipped in liquid ethane (EM GP, Leica Microsystems GmbH, Wetzlar, Germany) and transferred to a cryo holder (JEOL Ltd., Tokyo, Japan) for direct observation at −269°C in a JEM3000EFC (JEOL Ltd., Tokyo, Japan) containing a top-entry liquid-helium-cooled stage and operated at 300 kV using an MDS (Minimum Dose System, JEOL Ltd., Tokyo, Japan). Micrographs were obtained using a sensitive charge-coupled device camera (Temcam-F214, TVIPS GmbH, Gauting, Germany). By employing this observation method, the platelet structure could be observed from above, and the side views of the nanoplatelet stacks of the internal layered structure could be visualized as previously reported. The size distribution of the platelets, i.e., the length and width as measured from above, was also determined.

2.10 Measurements of oil migration

The extent of oil migration, which is one of the factors used to ascertain quality defects of fat spread products, was determined. Here, W/O emulsion samples were molded into a steel cylinder (10 mm in diameter and 5 mm thick). Then, the specimens were kept at 0°C for 30 min before a scaled filter paper (5 mm × 40 mm) was inserted into the center of the mix. The vertical oil penetration distance into the filter paper at 28°C was recorded after 90 min.

2.11 Observation by polarized light microscopy

Approximately 50 mg of the fat blend was placed on a glass plate heated to 80°C and covered with a glass coverslip. Glass coverslips that were ca. 30 µm thick were used as spacers to ensure uniformity of the sample thickness. Then, the samples were cooled and stored in an incubator at 10°C until analysis. A Leica DM2700P polarized light microscope (PLM) (Leica Microsystems, Tokyo, Japan) was employed to observe the microstructures of the bulk fat blends. The mean diameters of the fat crystals were calculated using image analysis software. When measuring the size of the spherulite, all pictures were converted to binary images to allow characteristic features to be distinguished from background interference in the software.

2.12 Statistical analyses

One-way analysis of variance was used to compare the mean values obtained for the groups. Post hoc multiple comparisons were conducted using Tukey’s test, with p < 0.05 considered statistically significant. The statistical analyses were performed using PASW Statistic 18 (SPSS Japan Inc., Tokyo, Japan).

3 Results and Discussion

3.1 The nanoplatelet structure of fat-functionalized SEFs

The cryo-TEM images and estimations of the crystal size of the nanoplatelets are shown in Fig. 1. The length and width of the crystals, as well as the length/width ratio, are shown in Table 1. The length of the L–SE crystals was not different from that of the control, whereas the length of the P–SE and S–SE crystals was shorter than that of the control. For the three esterified saturated fatty acids (namely, lauric, palmitic, and stearic acids), long carbon chains of the fatty acid resulted in short nanoplatelets. The length of the crystals for both O–SE and the control sample was similar, whereas the crystal length of ER–SE was smaller than that of the control sample. These observations indicated that longer carbon chains on the fatty acids esterified to sucrose resulted in shorter nanoplatelets for both the saturated and unsaturated fatty acids. Furthermore, the addition of the SH–SE blend caused a decrease in the crystal length; however, the extent of the decrease was less than that for the S–SE blend. The ratio of mono-, di-, tri-, and polyester of S-170 was 1:99, while that of S-370 was 20:80, indicating that the SH–SE blend was more hydrophilic than the S–SE blend due to its lower esterified rate of fatty acids. Therefore, the differences in the crystal sizes were attributed to the higher affinity of the SH–SE blend to the interface of the W/O emulsion than the S–SE blend. As such, the contribution of the SH–SE blend to the overall crystallization process was lower.

The differences in the width of the nanoplatelets were not more marked than the variations observed in the length of the nanoplatelets. The L–SE blend contained wider nanoplatelets than the control sample, whereas the S–SE blend contained narrower nanoplatelets. The addition of S–SE decreased both the length and the width of the resulting nanoplatelets. In contrast, the addition of lecithin did not influence the size of the nanoplatelets. The ratio of length/width for each blend is shown in Table 1. The length/width ratio decreased in the following order: control > L–SE > P–SE > S–SE for the saturated fatty acids, and control > O–SE > ER–SE for the unsaturated fatty acids. Thus, the addition of SEFs with long chain fatty acids produced a lower length/width ratio for the nanoplatelets.

3.2 Microstructure of bulk fat systems via polarized microscopy

Fat crystal networks are arranged hierarchically with characteristic nanoscale and aggregated mesoscale structures. These polycrystals are created by the agglomeration of TG nanoplatelets that constitute the primary crystals formed upon nucleation. To investigate these structures, bulk phase blends without any added water phase were prepared according to the requisite formulation, and these crystals were observed using PLM. The respective diameters of the spherulite were measured. SEFs
Fig. 1  Images of crystalline nanoplatelets and the associated histograms obtained from W/O semisolid fat blends monitored using cryo–TEM.
Above: Cryo–TEM photomicrographs of the fat blends’ nanoplatelets.
Below: The corresponding frequency size distributions for the various nanoplatelet lengths.
were added to the fat phase and significant changes were observed in the crystal morphology (Fig. 2). The size of the spherulite from bulk fats was much larger than that of the SEF-containing nanoplatelet samples prepared via W/O emulsion and subjected to rapid cooling (Table 1). The crystals of semisolid fat blends containing SEFs with saturated and long-chain fatty acids (namely, P–SE, S–SE, and SH–SE) were smaller than those associated with the control sample. While the smallest crystals were obtained for fats containing SEFs with a stearic acid moiety. No size difference was observed between the S-SE and the SH–SE blends. Next, the spherulite size was considered. The spherulite size in the L–SE blend was bigger than that of the control sample. Differences in spherulite size between the control and the O–SE blend were also observed. These results suggested that SEFs with long chain and saturated fatty acids reduced the size of the spherulites in the bulk fat system by directly affecting the triacylglycerol (TG) crystal formation. The crystal size of LE was smaller than that of the control sample, which was in accordance with previous studies conducted with a blend of palm oil and palm olein\(^7,22\), where it was reported that palmitic and stearic sucrose ester with HLB = 1 delayed nucleation, ultimately leading to smaller crystals.

Figures 3A and 3B show the relationship between the nanocrystal sizes (as observed using cryo–TEM) and the diameter of the microscale spherulite (as observed using PLM). The determination coefficient of the nanocrystal length and width relative to the spherulite diameter was calculated to be \( R^2 = 0.573 \) and \( R^2 = 0.625 \), respectively. A similar interdependent relationship existed between the size of the nanoplatelets in the W/O emulsion sample, which was subjected to rapid cooling, and the diameter of the spherulite in bulk fats. These results suggested that the molecules of SEFs (with hydrophobic nature) used in this study tend to exist in the oil phase in W/O emulsions. Therefore, in this study, it was clear that the size of the nanoplatelets influenced the degree of aggregation in the structures.

### 3.3 Determining the crystal structures using small- and wide-angle X-ray analysis

The long spacing, as determined using XRD analysis, corresponds to the thickness of the lamellae\(^10\). In this study, the thickness \( (d) \) was calculated using small-angle X-ray analysis (Table 1). Figures 3C and 3D show the correlation between the nanocrystal length and \( d (R^2 = 0.187) \), as well as the correlation between the nanocrystal width and \( d (R^2 = 0.188) \). The results suggest that the nanocrystal thickness was not influenced by the nanoplatelet length or width.

Palm oil is widely used as a hardstock ingredient in the manufacture of margarine. Palm oil-containing fat blends used in this study tend to cause polymorphic transitions.
Effect of Fatty Acids of Sucrose Ester on the Crystal Nanostructure

Fig. 2  Polarized light microscopy images of the bulk fat samples prepared using different additives.

Fig. 3  The relationship between the nanostructure extracted from the respective W/O emulsion fat blend and the microstructure of the bulk fat system.
A: The relationship between the nanocrystals’ length and the size of spherulite, B: The relationship between the nanocrystals’ width and the size of spherulite, C: The relationship between the nanocrystals’ length and the nanoplatelets’ thickness (d), and D: The relationship between the nanocrystals’ width and the nanoplatelets’ thickness (d).
from the β to the β form that are due to the segregation of the TG, 1,3-dipalmitoyl 2-oleoyl glycerol (PO). Fat blends containing fully hydrogenated rapeseed fat and palm oil tended to form β-type polymorphs after long storage periods. In addition, the presence of saturated monoacylglycerols and tripalmitoylglycerol in palm based fat-induced the formation of a polymorphic β form. Therefore, polymorphs (β/β') of samples were compared using wide-angle X-ray analysis. The β/β' ratio of the SH–SE blend and LE was higher than that of the other samples containing SEFs (Table 2), suggesting that palm-based fat, which contains a hydrophilic emulsifier, promoted transformation to a stable β form.

3.4 Effect of fatty acids moieties on the SFC

The effect of additives on the SFC was found to be dependent on certain base fat formulations. To understand how the presence of certain fatty acid moieties on SEFs could affect the degree of crystal formation, the effect of SEFs on the SFC of semisolid fat mixtures containing fat blend systems with monoacylglycerol was determined in this study (Table 2). We found that the SFC of the P–SE and S–SE blends was higher than that of the control sample between 5 and 35°C. The SFC of L–SE, LE, and SEFs with unsaturated fatty acids (O–SE and ER–SE) was the same as that of the control sample, but lower than that of the samples in which the SEFs were esterified with saturated fatty acids. Thus, the SFC increased in the following order: L–SE < P–SE < S–SE from 20°C to 30°C, indicating that the presence of SEFs with long fatty acid chains accelerated fat crystallization. While the SEFs with unsaturated fatty acids did not affect the SFC. Using SFC measurements, Herrera et al. reported that the addition of SEF with saturated fatty acids to O/W emulsion systems accelerated crystallization for the high-melting fractions of milk fat and its mixtures with sunflower oil. Domingues et al. noted accelerated crystallization was noted in the esterified fat of soybean oil and the fully hydrogenated soybean oil upon the addition of SEFs with stearic acid. Garbolino et al. showed that fatty acid residues of sucrose palmitate could interact to cause co-crystallization of the palmitic acid residue that resulted in higher SFC values; this study was conducted using a fat blend containing palm oil and fat esterified with palm kernel oil and sunflower oil.

3.5 The effect of the fatty acid moieties of SEF on the storage elastic modulus, G' **

The physical properties of the semisolid fat W/O emulsion are affected by the gelation of fat during the crystallization process. The temperature dependence of G' was determined for the semisolid emulsions containing SEF that had been prepare using the rapid cooling method (Fig. 4). The G' value of the samples was measured between 5 and 30°C, with an incremental increase of 0.5°C/min. The
The G’ value of L–SE was similar to that observed for the fat blend containing SEF esterified with unsaturated fatty acids (O–SE and ER–SE), whereas the G’ value of LE mirrored the trend seen for the SEF samples esterified with unsaturated fatty acids. Mid-length chain fatty acids had similar physical properties to the semisolid fat blends regardless of the associated saturated fatty acid moiety. Additionally, LE consisted of fractionated soybean lecithin that possessed unsaturated fatty acids; which could account for the similarity in the physical properties of SEF esterified with unsaturated fatty acids. For the saturated fatty acid samples, higher G’ values were noted above 25°C when compared to the values seen for the control sample; in contrast, when the temperature was below 25°C, the G’ value for all samples was lower than that of the control.

The G’ value of most samples decreased with increasing temperature from 5 to 15°C and from 15 to 20°C and then decreased again above 23°C. This characteristic temperature dependence was observed in the control sample. Lopez and colleagues reported an increase in G’ at approximately 20°C for dairy products; however, the cause was not identified. We suggest that this behavior in the control sample was due to a change in the polymorph of the TGs or reconstitution of solid fat crystals between 15 to 20°C, which is fixed in a 2 mm gap parallel plate in the rheometer. A combined investigation observing the microstructure of the solid fat network and monitoring the polymorph could verify our hypothesis.

Furthermore, the addition of emulsifiers with a high melting point to the fats was reported to increase the rate and extent of fat crystallization. In this study, a similar trend was observed in the W/O emulsion containing semisolid fats; thus, resulting in higher G’ values in the fat blends containing SEF esterified with saturated fatty acid moieties. This suggests that the physical properties are influenced by the interaction between the fat crystals.

3.6 Effect of the fatty acid moieties of SEF on oil migration
The excess oil from fatty food is of great concern to food manufacturers as it causes undesirable changes in the properties and function of fat-containing foods. Therefore, the effect of SEF esterified with various fatty acid moieties on oil migration for W/O emulsion semisolid samples was investigated (Table 2). We established that P–SE and S–SE had low oil migration tendencies when compared to the other samples. Additionally, there were no significant differences between S–SE and SH–SE regarding oil migration. Rouseau showed that the crystal network was attached to the water droplets in the W/O-type semisolid emulsion and that this behavior ultimately influenced the temperature-dependent character of oil migration. However, in this study, this influence was not observed statistically due to the low detection accuracy of our procedure.

3.7 Effect of the nanoplatelet size on the physical properties of the semisolid fat blend
The nanoplatelets, which were generated during the rapid cooling process through nucleation and crystal growth, immediately aggregated into larger units, leading to the creation of a three-dimensional crystal network structure. The network structure influences a variety of mechanical properties such as the storage modulus and oil migration of the fat products. Figure 5A shows the relationship between the crystal length and the SFC at 10, 25, and 30°C. Higher SFC values were seen with lower temperatures, whereas a decrease in the SFC value was observed when a corresponding increase in the crystal size was noted within a specific temperature range. Quantitatively, the relationship between the crystal length and the oil mi-
An increase in the length of the nanoplatelets at 25 and 30°C led to a decrease in SFC (Fig. 5A); however, this dependence was not observed at lower temperatures. The correlation between the SFC and the \( G' \) values at 10°C and 30°C was \( R^2 = 0.049 \) and \( R^2 = 0.884 \), respectively (Figs. 5C and 5D). The \( G' \) value did not show a linear relationship with the SFC in the samples prepared at 10°C. However, a close relationship between the two values was noted at 30°C. The lower correlation between the SFC and the \( G' \) values at 10°C was due to the higher \( G' \) value of the control at 10°C compared with the other samples. This result suggested that additives (SEF and fractionated lecithin) used in this study decreased the strength of the fat crystal network related to \( G' \), particularly in the case of abundant amounts of fat crystals. Therefore, the regulation of the physical properties of the semi-SFC by SEF was more effective at ambient conditions than when conducted under cold conditions.

The rheological properties of the semisolid fats were dependent on the ratio of crystallized fat to oil, and the structure of the crystallized fat. In general, higher SFC values promoted more solid-like behavior of fat products. The relationship between the SFC and the storage elastic modulus can be explained theoretically\(^3\). The oil migration in fatty food products is due to a combination of diffusion and capillary flow processes\(^38\). The nanoplatelets generated during the rapid cooling stage interacted with each other and aggregated into larger microstructures via van der Waals forces; thus forming a three-dimensional network. To this end, the Marangoni group studied the effects on the nanostructure of TG crystal networks. By comparing the nanostructure of several fat ingredients,
they showed that the nanoplatelet size appears to influence the physical properties.

In this study, we confirmed that the size of the generated nanocrystals and SFC significantly influenced the physical properties of palm-based W/O fats containing SEF with various fatty acids moieties. To verify the mechanism through which SEF exerts its influence on the physical properties, more detailed work is required in which a mass fractal model can be used to define the relationship between nanostructures and the supersaturation of each sample.

4 Conclusions

In this study, W/O emulsion semisolid fat blends with added SEF esterified with several fatty acid moieties were prepared via the rapid cooling method. The nature of the nanoplatelet structure was determined by X-ray analysis, and physical properties (elastic modulus and oil migration) were determined. Changes in the size of the nanoplatelets triggered by the addition of SEF esterified with various fatty acid moieties led to differences in the nanoplate size and physical properties. In addition, analysis of the microstructure of several bulk fat systems was conducted via PLM, and the SFC was also analyzed. The presence and type of SEF impacted both the nanoplate size in the W/O emulsion fat blends and the spherulite size in the fat blends, with similar tendencies in size. Smaller platelets generally resulted in higher SFC in bulk fats, and a high correlation between the SFC and G increase was observed at 30°C. Samples with larger nanocrystals had a higher propensity for oil migration. Thus, the addition of SEF regulated the fat crystal nanostructure during nucleation and crystal growth, ultimately influencing the physical properties. In conclusion, the results in this study could provide useful information for influencing the manufacturing process of fat products that contain less trans-fat products such as margarine and shortenings.

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