Tailoring the Textural Attributes of Butter Fat/Canola Oil Blends via *Rhizopus arrhizus* Lipase-Catalyzed Interesterification. 2. Modifications of Physical Properties

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The effects of *sn*-1,3 specific *Rhizopus arrhizus* lipase-catalyzed enzymatic interesterification on the physical properties of butter fat/canola oil blends [100% butter fat (w/w) to 60% butter fat/40% canola oil (w/w) (60:40)] were determined. Enzymatic interesterification (EIE) lowered and linearized the solid fat content (SFC) versus temperature profile of all noninteresterified (NIE) blends in the 5–40 °C range. The largest drops in SFC as a result of EIE occurred with native butter fat between 5 and 10 °C. Dropping points (DP) decreased 1–3 °C for all blends upon interesterification, and the decrease in DP as a function of canola oil proportion in the blend was more pronounced for the EIE than for the NIE blends (0.16 °C/wt % versus 0.10 °C/wt %, respectively). Isothermal crystallization behavior monitored turbidimetrically (onset of crystallization) showed that EIE did not alter the crystallization behavior of butter fat, yet did alter that of the blends. Rheological characterization included large and small deformation tests. Cone penetrometry, used to gauge the former, indicated that both blending and EIE diminished the hardness index (HI) in a nonlinear fashion. Reductions in HI resulting from EIE predominantly occurred within 12 h of interesterification. Oscillatory frequency sweep measurements at small amplitude showed that EIE blends had lower storage (G') and loss moduli (G'') than their NIE counterparts. Blending of butter fat with canola oil and subsequent interesterification modified the crystal morphology and X-ray diffraction patterns of butter fat. Polarized light microscopy revealed that addition of canola oil led to slight aggregation of the crystal structure, whereas EIE led to more pronounced aggregation of the crystalline mass. X-ray diffraction analysis revealed a predominance of β' and a small proportion of β crystals for all samples examined with greater presence of β crystals in the EIE blends vis-à-vis the NIE blends. Long spacings increased from ~41.5 to 44 Å following EIE. The fractal dimension of the butter fat/canola oil blends diminished from 2.59 to 2.50 following interesterification. These results suggest that changes in the rheological properties of butter fat induced by enzymatic interesterification were closely linked to changes in the SFC and, to a lesser extent, to the distribution of crystal mass within the fat crystal network.

Keywords: *Rhizopus arrhizus; butter fat; canola; interesterification; lipase-catalyzed; oil*

INTRODUCTION

Recently, our group has examined the effects of chemical interesterification (CIE) on the physical and compositional properties of butter fat/canola oil blends (Rousseau et al., 1996a–c; Marangoni and Rousseau, 1996). CIE led to a significant decrease in the hardness of butter fat/canola oil blends (Rousseau et al., 1996c) and an improvement in cold-spreadability (Rousseau et al., 1998). However, this process affected butter flavor adversely (Weihe, 1961). An alternative to this process is lipase-catalyzed interesterification, which operates under milder reaction conditions.

Limited research has been performed on the effects of enzymatic interesterification (EIE) on the physical properties of butter fat. Kalo and co-workers (Kalo et al., 1986a,b, 1990) have examined the melting and crystallization properties of EIE butter fat using low-resolution pulsed NMR and/or DSC. His group has shown that there are few differences between chemically and enzymatically interesterified (with a nonspecific lipase) butter fat, both processes leading to similar changes in chemical composition and melting properties. Bornaz et al. (1994) studied ways of increasing butter solid fat content (SFC) at room temperature via addition of tripalmitin or trimyristin and EIE or a combination thereof. Oba and Witholt (1994) used a lipase from *Rhizopus oryzae* to interesterify milk fat and oleic acid. The proportion of high-melting fraction dropped substantially following modification. Foglia et al. (1993) studied the melting properties of EIE butter fat/sunflower blend properties. Mohamed and Larsson (1994) studied the polymorphic behavior of EIE fats, in their case, mixtures of fully hydrogenated soybean oil/cottonseed oil mixtures. Their results showed that the β' polymorph was stabilized following interesterification.

Since the 1970s, much work has been published on the enzymatic modification of fats and oils. Research has predominantly concentrated on the changes in composition following lipase-induced modification. For the successful use of such methods in the fabrication of tablespreads, an understanding of the changes in the

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physical properties following EIE is a prerequisite. In this research, we report on the melting, crystallization, rheological, and crystal properties of butter fat and butter fat/canola oil blends following interesterification with an *sn*-1,3 specific lipase from *Rhizopus arrhizus*.

MATERIAL AND METHODS

Source Materials and Preparation. Sample preparation and the EIE procedure are described in a previous paper (Rousseau and Marangoni, 1998). Non-interesterified is abbreviated NIE and enzymatically interesterified, EIE. Blends are identified as BF (butter fat) and 90:10 to 60:40 [90:10% to 60:40% (w/w) butter fat/canola oil blend].

Removal of Partial Acylglycerols and Free Fatty Acids (FFA). Partial acylglycerols and FFA are known to influence physical properties. We examined the effects of diacylglycerol (DAG), monoacylglycerol (MAG), and FFA removal from an 80:20% butter fat/canola oil blend. The samples were treated with methanol five times and the methanol phase extracted. Chromatographic analysis was performed according to the procedure of Rousseau and Marangoni (1998).

Pulsed Nuclear Magnetic Resonance. Solid fat content (SFC) was measured by pulsed nuclear magnetic resonance (pNMR) using a Bruker PC/20 Series NMR analyzer (Minispec, Milton, Canada) (AOCS official method Cd 16-81). The samples were then sequentially conditioned for 30 min from 5 to 45 °C at 5 °C intervals.

Isothermal Crystallization Behavior. Crystallization studies were performed to determine the crystallization onset temperature and rate by changes in optical density at 490 nm (Chaiseri and Dimick, 1995; Grall and Hartel, 1992). Samples (2 mL) were placed in NMR tubes, refrigerated for 24 h at 5 °C in a water-filled container, and then heated for 5 min at 60 °C in a thermostated water bath. They were subsequently transferred to a cuvette placed in the cuvette holder of a temperature-controlled spectrophotomer (Beckman DU-7000 Series spectrophotomer) maintained at 10 °C by a circulating waterbath. Readings at 15 s intervals were taken until optical density readings were off-scale. Standard blank was a clear sample of the sample being analyzed.

Dropping Point Determination. Dropping points were measured using the Mettler dropping point apparatus. The instrument, model FP83 (Mettler, Zurich, Switzerland), contains a sample cup with an opening at the bottom through which a sample flows and sections an IR beam, which is then read as the DP. Liquefied samples were pipetted into chilled sample holders and held at −20 °C for 1 h before measurements were initiated 12–15 °C below the expected dropping point. A heating rate of 1 °C/min was used.

Cone Penetrometry. A cone penetrometer (AOCS Method Cc 16-60) was used to decide the hardness index (HI) of the blends. The penetrating cone (m = 92.5 g) was placed just above the surface of the sample and released. The penetration depth in 0.1 mm increments was read on the dial after 5 s of contact. Analyses were performed in a refrigerator maintained at 5 °C. Five replicates were performed for each sample. Penetration readings of 15–150 units were considered a suitable range for conversion to a rheological property (Hayakawa and deMan, 1982). The following equation was used to convert the penetration data into hardness index (HI):

$$HI = \frac{\text{mass of cone (g)}}{\text{depth of penetration (mm)}} \times 0.1$$

Controlled Stress Rheometry. Dynamic oscillatory measurements were conducted using a Carri-Med CSL^2 500, controlled stress rheometer (TA Instruments, New Castle, DE) using a 2 cm parallel plate geometry with a solvent trap. Sample slippage was minimized by attaching 50 grit sandpaper to both the upper flat fixture and the Peltier base.

Liquefied samples were crystallized in a three-piece PVC mold assembly consisting of a top and bottom plate and a midplate (h = 3200 μm, d = 2 cm). The mold was securely fastened together with screws. Care was taken to avoid formation of air bubbles. Sample compression was minimized to prevent sample deformation yet sufficient to achieve good contact between the sample and constraining surfaces (calculations not shown). The molds were incubated in a refrigerator for 24 h at 5 °C. Triplicate analyses were performed for all samples.

The linear viscoelastic region (LVR) was determined at 1 Hz with a torque sweep of 0.64–150 Pa. The LVR range observed was approximately 5.0×10^{-5} to 1.0×10^{-3} rad, after which irreversible sample damage or slippage occurred. For sufficient instrument sensitivity, a minimum displacement of 1.5×10^{-4} radians was judged to be necessary.

A frequency sweep of 0.1–10 Hz at a displacement of 1.5×10^{-4} radians was performed at 5.0 ± 0.1 °C. To reduce heating from the surroundings, the fixture, solvent trap, spacer, and all utensils used to handle the fat were cooled in ice water before use.

Dynamic oscillatory test parameters, G' and G'', were calculated using the software provided by the manufacturer.

Polarized Light Microscopy. A small amount of fat was placed on prechilled viewing slides (Fisher, St. Louis, MO) to which was added a drop of paraffin oil (Fisher), a dispersing aid. A cover slip (Fisher) was then firmly pressed on the sample to remove air bubbles and excess liquid. An Olympus BH polarized light microscope (Tokyo, Japan) mounted with a PM-6 35 mm camera was used. Visual magnification was 250× (10× ocular and 25× lens). Photomicrographs were taken on Kodak T-Max 100 ISO film with 20 s exposure times. Samples were maintained at 10 °C by a cold stage temperature-controlled by a circulating water bath. Many samples were examined, and each photomicrograph represents a typical field.

X-ray Diffraction Spectroscopy. The polymorphic modifications of the NIE and EIE butter fat and the 90:10 to 70:30 blends as well as NIE60:40 were determined by powder X-ray diffraction. Samples were liquefied at 70 °C for at least 10 min in a water bath to remove crystal history. Using a pipet, 20 μL was put on the ridge of a curved glass plate (r = 2.5 cm, l = 4 cm). The sample solidified as film on the ridge of the plate at room temperature. These were then refrigerated at 4 °C for at least 24 h before analysis. Samples were held at 5 °C by the temperature-controlled chamber while the diffraction pattern was collected.

The X-ray beam was generated by a Elliot GX6 rotating anode machine operating at 30 kV and 20 mA. A line focus was used, and the beam was 500 μm thick and ~0.5 cm wide. The monochromatic Cu Kα line was produced using a combination of a nickel-coated Franks mirror and a nickel filter. The line was oriented at a grazing angle with respect to the film on the curved glass plate. The diffraction patterns were recorded with a model B-OED-50S (M. Braun, GmbH, Munich, Germany) linear position sensitive detector, which had an effective length of 50 mm and the appropriate timing electronics. The output from the timing electronics was from a time to amplitude converter. The diffraction pattern was obtained from this signal using a multichannel analyzer (Aptec Engineering, Mississauga, ON) housed in a microcomputer. The detector was filled with 95% Ar and 5% CH$_4$ at a pressure of 11.5 bar and held at a potential of 3.5 kV. The sample to detector distance was ~15 cm. The system was calibrated using crystalline potassium hydrogen phthalate, which has strong reflections at 1.34 and 0.498 nm. The samples were held in a specially constructed chamber. The temperature was controlled by a water bath, which circulated liquid through channels cut in the base plate of the sample chamber. The thick-walled brass chamber had 50 μm Mylar windows for the X-ray beam.

Sample Measurements and Statistical Analysis. Triplicate analyses were performed on blends and EIE replicates for all pNMR, isothermal crystallizations, DP, and controlled stress rheometry measurements. Values reported are averages and standard errors. CP results are based on quintuple measurements. Statistical analysis was performed using the
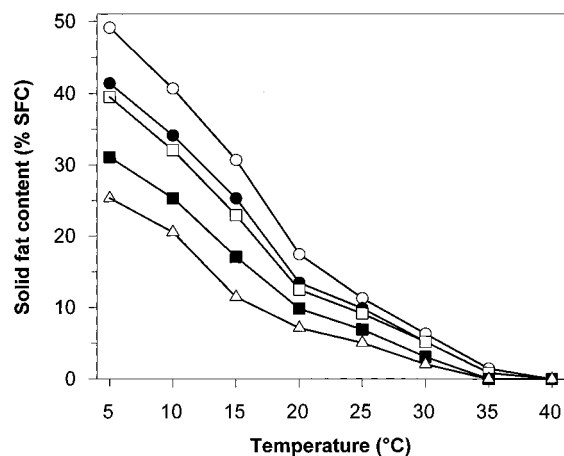
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Figure 1. SFC evolution of butter fat/canola oil blends as a function of temperature (°C): NIEBF (○); NIE90:10 (●); NIE80:20 (□); NIE70:30 (■); NIE60:40 (△).
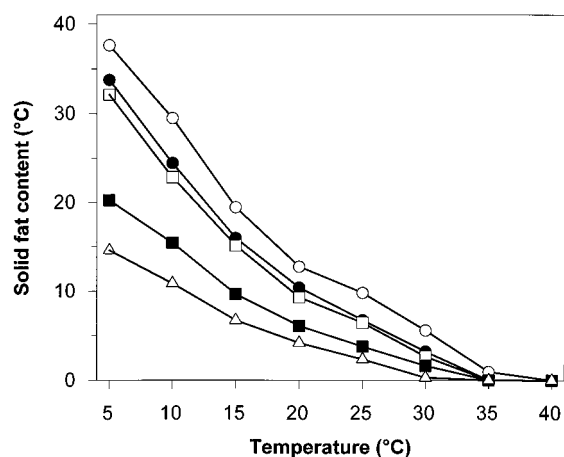

Figure 2. SFC evolution of EIE butter fat/canola oil blends as a function of temperature (°C): EIEBF (○); EIE90:10 (●); EIE80:20 (□); EIE70:30 (■); EIE60:40 (△).

SAS General Linear Methods (GLM) procedure (SAS Institute, 1994). Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Melting and Crystallization Properties. *SFC.* The SFC of all interesterified blends was consistently lower than that of the NIE blends at all temperatures measured. Figure 1 shows changes in SFC as a function of temperature for NIE blends ranging from NIEBF to NIE60:40. A sharp drop in SFC occurred between 15 and 20 °C for NIEBF, NIE90:10, and NIE80:20. Above 20 °C, the decreases in SFC as a function of increasing temperature were less pronounced. Addition of canola oil progressively lowered SFCs at all temperatures and somewhat linearized the melting profile. EIE of the blends (Figure 2) caused a drop in SFC at all temperatures relative to NIE samples and altered melting profiles. The largest drops in SFC as a result of EIE occurred at 5 and 10 °C for native butter fat and for the 60:40 and 70:30 blends. Between 10 and 20 °C, differences in SFC between NIE blends and their interesterified counterparts decreased such that, above 20 °C, the differences in SFC were small. These results are similar to those reported by Foglia et al. (1993), who enzymatically interesterified a 50:50% butter fat/sunflower oil blend and noticed a drop in SFC (measured between −10 and 30 °C) following EIE. However,
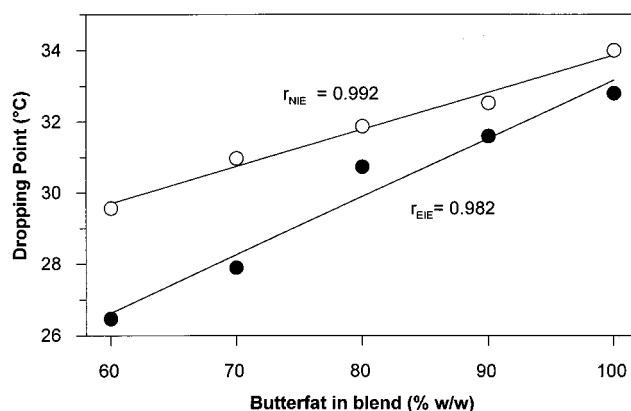

Figure 3. DP evolution (°C) of NIE and EIE butter fat/canola oil blends as a function of percent butter fat (w/w) in the blend: NIE blends (○); IE blends (●).

changes in SFC induced by EIE are different from those induced by chemical interesterification (Rousseau et al., 1996a; Becker, 1959). Chemical interesterification leads to decreases in SFC below 15 °C (as does EIE) but to increases in butter fat SFC from 15 or 20 °C to end of melt. These profound differences between EIE and CIE are due to differences in the nature of the triglyceride structure retailoring process.

Furthermore, our results conflict with those of other researchers who have enzymatically interesterified butter fat with either random or regiospecific lipases. Bornaz et al. (1994) increased the SFC (measured by DSC) of butter fat from ~21 to ~40% (at 20 °C) following 10 h of EIE with an *sn*-1,3 specific lipase. Kalo et al. (1990) found that the SFC (at 20 °C) of butter fat interesterified at 50 °C with an *sn*-1,3 specific lipase in the absence of solvent increased from 21.3 to 30.3% compared with untreated butter fat. Reactions were carried out for 8−10 days, however, compared with only 24 h for our system. Kalo et al. (1986b) enzymatically interesterified a butter fat solid fraction/rapeseed oil blend to discover that the latter has a narrower melting range than the former. Globally, EIE had a highly significant effect on SFC ($P < 0.0001$), and there was also a highly significant interaction effect between interesterification and blend composition ($P < 0.0001$).

The presence of non-triacylglycerol (TAG) species did not noticeably influence the melting behavior of the 80:20% butter fat/canola oil blends, as determined by SFC measurements. Methanol washing decreased both MAG and DAG species present in the samples, while reducing the proportion of FFAs from ~4.4 to ~2.1%. Consequently, other samples were not subjected to a methanol wash.

Dropping Point (DP). Figure 3 shows the effects of EIE on the DPs of butter fat/canola oil blends (100 to 60% butter fat). The decrease in DP as a function of blend composition was more pronounced for the EIE blends than for the NIE blends (0.16 °C/wt % versus 0.10 °C/wt %, respectively). DPs dropped by ~4.4 °C between NIE100 and NIE60 blends, whereas for EIE blends, the drop was 6.3 °C. The blend DP temperature difference between NIE and EIE blends increased from 1.2 °C for 100% butter fat to 3.1 °C for the 60:40% blend. EIE of a 50:50% butter fat/sunflower blend has been reported to increase its melting point, even though the SFCs were lower from −10 to 30 °C (Foglia et al., 1993). Larger changes in DP for EIE blends can be attributed to either the large decrease in SFC or the rearrange-
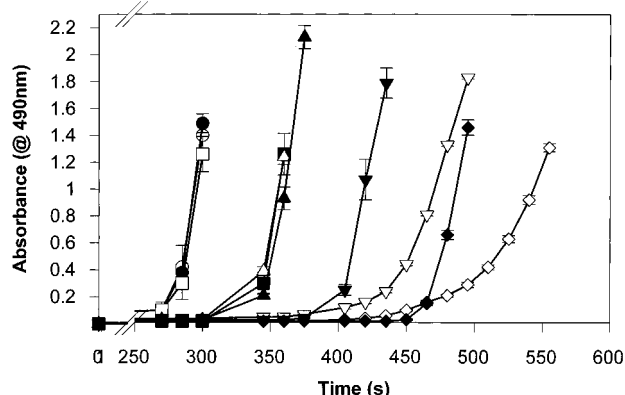
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Figure 4. Isothermal crystallization (at 10 °C) measured by optical density ($\lambda = -490$ nm) of NIE and EIE butter fat/canola oil blends: NIE100 (○), EIE100 (●); NIE90:10 (□), EIE90:10 (■); NIE80:20 (△), EIE80:20 (▲); NIE70:30 (▽), EIE70:30 (▼); NIE60:40 (◇), EIE60:40 (◆).

Figure 5. Effect of interesterification duration on hardness index (g/mm) of butter fat/canola oil blends as a function of percent butter fat (w/w) in the blend: BF (○); 90:10 blend (●); 80:20 blend (□); 70:30 blend (■); 60:40 blend (△).

ment of the fat crystal structure (shown later) resulting from changes in TAG distribution after interesterification. Incorporation of unsaturated fatty acids from canola oil into butter fat TAGs results in a disruption of crystal structure, leading to a lower melting temperature (Norton et al., 1985).

The SFC value corresponding to the DP was between 1.7 and 3.2% for all blends, suggesting that little crystalline matter was necessary to maintain sufficient cohesion to trap liquid oil within the crystalline matrix. These SFC values at the dropping point temperature agree with previously reported values (deMan et al., 1983). All DPs were significantly different from each other ($P < 0.0015$).

Crystallization Curves. Isothermal crystallization curves for all NIE and EIE blends are shown in Figure 4. Nucleation can be defined as a chain reaction advancing through a multitude of clusters with the result that some of them reach macroscopic dimensions (Toschev, 1973). The increase in observed optical density can be the result of either nucleation or crystal growth but probably is a combination of both (Boistelle, 1988). For purposes of statistical analysis, the crystallization onset was taken as the time at which the absorbance exceeded 0.1. Under the conditions used, no statistically significant differences in crystallization behavior were observed between NIEBF, EIEBF, and NIE90:10 ($P > 0.05$). Onset of crystallization for these blends occurred at ~250 s, and the crystallization process progressed rapidly. Crystallization onset for the EIE90:10, NIE80:2), and EIE80:20 blends was ~325 s, and no significant differences in onset of crystallization were detected among these samples ($P > 0.05$). The onset of crystallization for the 70:30 and 60:40 NIE blends occurred earlier, and the crystallization process progressed more slowly than for their EIE counterparts. These results suggest that EIE did not significantly affect the crystallization behavior of native milk fat. The effect of EIE on the crystallization behavior of butter fat/canola oil blends was not clear. Techniques other than turbidimetry would have to be utilized to determine the mechanism by which EIE affected crystallization behavior in these blends.

Rheology. *Cone Penetrometry.* The HIs for NIE and EIE blends as a function of the amount of butter fat (w/w) present and EIE duration are shown in Figure 5. Most of the changes in HI for all samples occurred
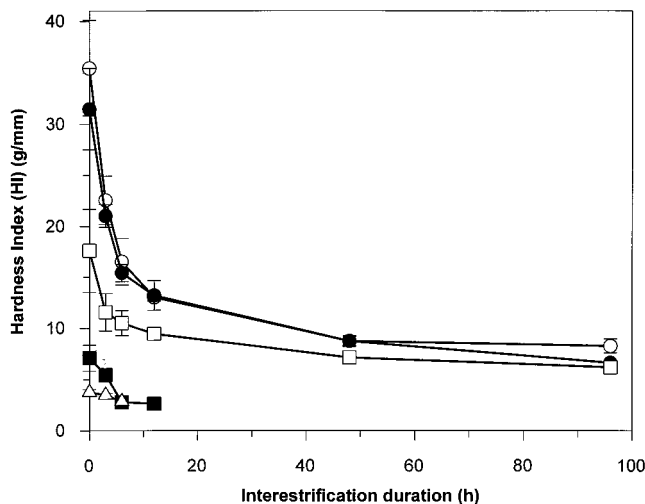
within 12 h of EIE. The HI of 100% butter fat dropped 34% following 3 h of EIE and 63% after 12 h. A similar pattern was observed for the 90 and 80% butter fat blends, which both underwent ~30% decreases in HI following 3 h of interesterification.

The rapid change in hardness suggested that only limited structural rearrangement was necessary to induce large changes in rheological properties (Rousseau and Marangoni, 1998). These results complemented those of the SFC evolution as a function of interesterification duration, which indicated that the largest changes in SFC occurred within 3 h of EIE (results not shown). For the blends examined, the softening effect produced by addition of liquid oil only was probably caused by dissolution of solid TAGs in the added canola oil, leading to a lower SFC at a given temperature (Timms, 1978). This may explain the nonlinear change in HI of the NIE blends as a function of the proportion of canola oil added.

Other factors influencing the HI of blends may include the modification, albeit small, in the proportion of long-chain trisaturates present in the butter fat, which are influential on rheology of butter fat (Simoneau et al., 1994).

Controlled Stress Rheometry. Small deformation dynamic oscillatory testing provides relevant information regarding the structure of the fat crystal network (Davis, 1973). The parameters measured were G' (storage modulus), which gives an indication of the solidlike or elastic component, and G'' (loss modulus), which provides an estimate of the fluidlike viscous behavior of a viscoelastic material (Ferry, 1970). Table 1 shows the effect of gradual replacement of butter fat with canola oil and/or EIE on the storage (G') and loss (G'') moduli of the blends. For all systems examined, G' was substantially higher than G'' (Rousseau et al., 1996c; Shukla and Rizvi, 1995; Drake et al., 1994). Even though the solid fraction necessarily contributes the majority of the structural network in plastic fats, fats with similar SFCs can have very different textural properties (Rousseau et al., 1996c; Haighton, 1976). For example, the SFCs (at 5 °C) for NIE80:20 and EIEBF were very close at 39.5 and 37.6%, respectively. Rheological data showed, however, that the G' of EIEBF was half that of NIE80:20. However, the opposite trend was

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Table 1. Effect of Blending and EIE on Storage (G) and Loss (G') Moduli (at 1.14 Hz) of Butter Fat/Canola Oil Blends[a]

	NIE		EIE	
blend	G ($\times 10^5$ Pa)	G' ($\times 10^5$ Pa)	G ($\times 10^5$ Pa)	G' ($\times 10^5$ Pa)
100	70.78	13.81	26.57	6.31
90	52.2	12.18	18.17	4.3
80	47.67	10.21	11.57	4.3
70	23.46	4.66	2.92	0.89
60	4.96	1.7		

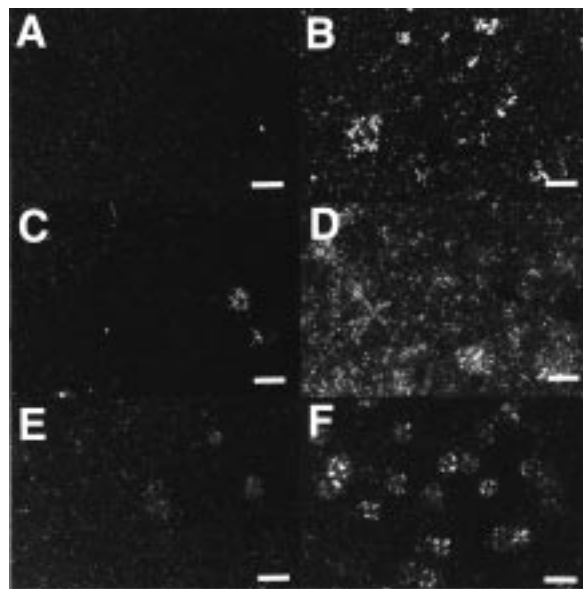[a] Values are averages based on duplicate determinations.

Figure 6. Polarized light microscopy photomicrographs of samples tempered for 24 h at 5 °C: (A) NIEBF; (B) EIEBF; (C) NIE90:10; (D) EIE90:10; (E) NIE80:20; (F) EIE80:20. The bar represents 25 μm.

observed for HI, with EIEBF's HI being higher than NIE80:20's.

Both blending and interesterification had significant effects on G' and G'' ($P < 0.0001$). There was also a significant interaction between blend composition and interesterification on G' and G'' ($P < 0.0001$).

Crystal Habit. *Polarized Light Microscopy.* EIE altered the crystal morphology of all blends. Figure 6A shows the small needlelike crystals found in butter fat following 24 h of tempering at 5 °C with no signs of spherulitic aggregation. On the basis of size alone, these are β' crystals, which normally measure <5 μm (Sato, 1988). Interesterification of butter fat induced changes in crystal morphology (Figure 6B), where many large spherulites were scattered among small crystals. These results agree with previous work (Rousseau et al., 1996b; deMan, 1961). The presence of canola oil in either NIEBF or EIEBF blends did not alter their

respective crystal morphologies. The differences observed between NIE and EIE samples may also be due to differences in the kinetics of recrystallization/aggregation. The samples examined in this study were viewed after only 24 h of tempering at 5 °C. Foley and Brady (1984) showed that a sample of milk fat crystallized for 7 days at 5 °C contained very large spherulites, not unlike those found in the EIEBF image. Hence, it is possible that the NIE butter fat examined in this study would rearrange to a similar state after a few more days of tempering. Overall, EIE led to the formation of spherulites, whereas blending alone resulted in little changes in crystal habit.

Distinguishing polymorphic forms solely on the basis of crystal habit is difficult as, visually, many morphologies can exist for a single polymorph (Kellens et al., 1992; Chapman, 1965). However, on the basis of crystal size alone, it would appear that the small platelets visible in each image are β' crystals. The large agglomerates may be simply groupings of β' or β crystals.

X-ray Diffraction. The long and short spacings of the NIE and EIE blends were determined with powder X-ray diffraction. X-ray diffraction patterns of milk fat produce broad peaks due to the large proportion of oil present in the samples, the small size of crystals, and the probable interchain disorder (Timms, 1980). Table 2 shows the short spacings and long spacings for the examined samples. The spacing at ~4.6 Å is associated with β-form crystals, while the others are associated with β' crystals (Larsson, 1966). All samples were combinations of β and β' crystals. Gradual replacement of butter fat with canola oil increased the relative proportion of β crystals. The presence of canola oil is known to promote the $\beta' \rightarrow \beta$ transition (deMan et al., 1995; deMan, 1989). EIE had the opposite effect to CIE on butter fat polymorphism. It has been shown by many (Rousseau et al., 1996b; Timms, 1979; deMan, 1961) that the β crystal form in butter fat is lost upon CIE. This is usually explained by the randomization of all fatty acids among all available glycerol binding sites. With an *sn*-1,3 specific lipase promoting interesterification, however, there was an increase in the proportion of β crystals. This may be interpreted as a loss of mixed crystals present in butter fat (Walstra and Beresteyn, 1975; Mulder, 1953). Mixed crystals incorporate disparate fatty acids into an imperfect matrix. EIE probably altered TAG structure in a way that mixed crystal formation was less likely to occur. This effect may partly explain the observed drop in SFC upon EIE. The changes in proportions of β' and β crystals were very small for the EIE blends compared to those for the NIE blends.

Long spacings were also examined in this study. Work by Timms (1980) showed that butter fat had long spacings of 42 and 65 Å. Our work showed long spacings of 41.5 ± 1 Å for all NIE blends. Interesteri-

Table 2. Short and Long Spacings of NIE and EIE Butter Fat/Canola Oil Blends

blend	long spacings (Å)	short spacings (Å)
NIEBF	42.0 (vvs), 20.8 (vw), 13.6 (w)	3.73 (w), 4.00 (s), 4.33 (vs), 4.64 (m)
NIE90:10	40.8 (vvs), 20.3 (vw), 13.5 (w)	3.73 (w), 4.00 (s), 4.36 (vs), 4.64 (m)
NIE80:20	42.7 (vvs), 20.8 (vw), 13.6 (w)	3.73 (w), 4.01 (s), 4.36 (vs), 4.64 (m)
NIE70:30	41.4 (vvs), 20.8 (vw), 13.6 (w)	3.73 (w), 4.01 (s), 4.36 (vs), 4.64 (m)
NIE60:40	42.0 (vvs), 20.8 (vw), 13.7 (w)	3.73 (w), 4.02 (s), 4.43 (vs), 4.68 (m)
EIEBF	45.4 (vvs), 22.8 (w), 14.7 (w)	3.92 (s), 4.29 (vs), 4.61 (m)
EIE90:10	44.6 (vvs), 23.5 (w), 14.4 (w)	3.85 (s), 4.29 (vs), 4.61 (m)
EIE80:20	44.6 (vvs), 22.8 (w), 14.3 (w)	3.84 (s), 4.25 (vs), 4.61 (m)
EIE70:30	44.6 (vvs), 23,7 (w), 14.2 (w)	3.84 (s), 4.26 (vs), 4.61 (m)

fication increased the first-order long spacings by 2−3 Å for all blends. These long spacings corresponded to a double-layer TAG structure (β'-2). deMan (1961) also examined long spacings and obtained similar data resulting from the CIE of milk fat. The reason for the increase in long spacings is unclear. What is clear, however, is that the change in long spacings was strictly associated with interesterification as the addition of canola oil did not alter the short and long spacings of the crystalline butter fat. This change in long spacings may be due to variations in fatty acid tilt (Kodali et al., 1984). Changes in tilt of the chain with respect to the end group plane can shorten the observed long spacing (Lutton, 1950). It is possible that longer unsaturated fatty acids are replacing shorter saturated fatty acids, leading to a combined effect of chain replacement and tilt.

Fractal Analysis. Fat crystal size and shape (presumably linked to polymorphism) are often considered the determining factors affecting rheological properties (Juriaanse and Heertje, 1988; deMan, 1987). For butter fat, however, physical properties are not necessarily dependent on polymorphism, as shown here and elsewhere (Rousseau et al., 1996a−c; Marangoni and Rousseau, 1996; deMan, 1964). Furthermore, it has been shown that samples with similar SFCs (1−2% difference) can have substantially different rheological properties. Given that the spreads examined were all relatively similar mixtures of β' and β crystals, the actual structure of the three-dimensional network of crystals and the interaction between the solid and liquid TAGs probably strongly influence the rheological behavior of the butter fat/canola oil blends.

Marangoni and Rousseau (1996) proposed the use of fractal geometry to characterize the relationship between fat crystal network microstructure and the rheological properties of spreadable fats, on the basis of an original study by Vreeker et al. (1992). Using the same approach in this study, the effects of EIE on the fractal dimension (D) of the butter fat crystal network were determined. To do so, a linear relationship between the logarithm of the SFC and the logarithm of G' (at 1.14 Hz) for NIE and EIE butter fat/canola oil blends (at 5 °C) was established

$$\log G' \sim m \log \text{SFC}$$

where m represents the slope derived by linear regression. For our system, the slopes of these lines are related to the fractal dimension of the butter fat crystal network in the weak link regime by

$$m = (d - 2)/(d - D)$$

where d is the Euclidean dimension (i.e., 3) and D is the fractal dimension of the fat crystal network (Shih et al., 1990).

The fractal dimension (D) of the butter fat crystal network diminished from 2.59 to 2.50 as a result of EIE. This drop in D is smaller than that induced by CIE (2.46 to 2.15) (Marangoni and Rousseau, 1996). Changes in D are normally attributed to changes in the structure of the fat crystal network. EIE caused a small reduction in D, suggesting only a slight change in the fat crystal network structure; however, a large reduction in SFC upon EIE was observed. In contrast, CIE induced a large structural change in the fat crystal netowrk (i.e.,

a large drop in D was observed), yet only slight changes in SFC (3−4% SFC) were observed.

During CIE, all fatty acids are theoretically randomized among all three glycerol sites, leading to a more homogeneous fatty acid distribution. This randomization leads to a restructuring of the fat crystal network, which in turn leads to a decrease in fractal dimension. EIE using a lipase displaying long-chain fatty acid and *sn*-1,3 specificity neither alters the fatty acid composition at the *sn*-2 site nor alters the positional distribution of short- and medium-chain fatty acids in butter fat TAGs. The more limited structural retailoring of TAGs upon EIE relative to CIE would result in less pronounced modifications in fat crystal network structure. The large drop in SFC induced by EIE could be due to mass lost upon hydrolysis, as well as specific changes in TAG structure which would impair the ability of TAG to form a mixed crystal lattice. Further work is required before a potential mechanism can be proposed.

This work has shown that 1,3-specific lipase-catalyzed interesterification is a valuable tool which can be used for the modification of the rheological properties of butter fat. EIE leads to decreases in the hardness and solid-like character of butter fat by inducing a large decrease in the amount of solids present and a small change in the structure of the fat crystal network. These results are in contrast with former work on random CIE by our group, in which CIE also induced decreases in the hardness and solid-like character of butter fat, however, via a different mechanism—CIE did not alter the amount of solid fat present but caused a pronounced change in fat crystal network structure. The rheological properties of butter fat can be modulated by alterations in solid fat content and/or fat crystal network structure.

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