

# Restructuring Butterfat Through Blending and Chemical Interesterification. 1. Melting Behavior and Triacylglycerol Modifications

Dérick Rousseau, Karine Forestière, Arthur R. Hill, and Alejandro G. Marangoni\*

Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

**ABSTRACT:** Chemical interesterification of butterfat–canola oil blends, ranging from 100% butterfat to 100% canola oil in 10% increments, decreased solid fat content (SFC) of all blends in a nonlinear fashion in the temperature range of 5 to 40°C except for butterfat and the 90:10 butterfat/canola oil blend, whose SFC increased between 20 and 40°C. The sharp melting associated with butterfat at 15–20°C disappeared upon interesterification. Heats of fusion for butterfat to the 60:40 butterfat/canola oil blend decreased from 75 to 60 J/g. Blends with >50% canola oil displayed a much sharper drop in enthalpy. Heats of fusion were 30–50% lower on average for interesterified blends than for their noninteresterified counterparts. Both noninteresterified and interesterified blends deviated substantially from ideal solubility, with greater deviation as the proportion of canola oil increased. The change in the entropy of melting was consistently higher for noninteresterified blends than for interesterified blends. Chemical interesterification generated statistically significant differences for all triacylglycerol carbon species (C) from C<sub>30</sub> to C<sub>56</sub>, except for C<sub>42</sub>, and in SFC at most temperatures for all blends.

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Growing consumer demand for healthy fat spreads that are palatable, can be used for cooking, and possess good textural properties has been the impetus behind the production of modified butters and butter-based spreads. Internationally, many products are present on the market, and new spreads are constantly emerging (1).

Over the last two decades, world consumption of butter has steadily declined due to many factors, ranging from butter's poor nutritional properties to its inherent physical limitations (2,3).

Butterfat is a mixture of more than 100,000 different triacylglycerols (TAG) with a melting range from –40 to 40°C (4). Jensen (5) noted the presence of ~400 different fatty acids (FA) in butterfat, 25% of which were short-chained and 45%

were long-chained saturates (6). Such variety in composition is responsible for butterfat's unique physical properties (7).

Contrary to margarine, butter has a limited plastic range (7). At refrigerator temperature (~10°C), butter essentially behaves as a solid and lacks spreadability, whereas at room temperature (21–25°C), it oils off and exhibits moisture exudation. Butter's "ideal" spreadability is at ~15°C when its solid fat content (SFC) is around 30% (2).

Methods used to improve butter spreadability include thermal fractionation of butterfat, treatment of the cream, and blending with oil. In Sweden, the pioneering country for manufacture of blended fat spreads, a low-fat ripened cream-soybean oil blend, Bregott, was introduced in 1973 (2).

Nutritionally, butterfat contains a high percentage of hypercholesterolemic fatty acids (mid-length saturated FA) located predominantly at the *sn*-2 position of TAG (8). Conversely, it contains only a small percentage of *trans* fatty acids. Thus, it has avoided the backlash associated with partially hydrogenated margarines.

Blending butterfat with vegetable oils that are liquid at refrigerator temperature can lead to spreads that harmonize nutrition, and offer desirable organoleptic attributes and lowered overall costs of production.

The premier cash crop in Canada is canola oil, a rapeseed oil low in erucic acid and high in oleic acid, making it an ideal choice for incorporation into edible fat spreads. It is also essentially odorless and flavorless, and thus will not affect the fine flavor so desirable in butter.

Another means of modifying fat structure is interesterification. Two types of interesterification are in commercial use—chemical and enzymatic. While enzymatic interesterification may represent the way of the future, at present, chemical interesterification is a cheaper process that is tried-and-true on an industrial scale.

Chemical interesterification causes a statistical randomization of FA distribution that leads to modifications in TAG composition and, consequently, in physical behavior. As interesterification progresses, FA moieties are shuffled within (intraesterification) and among (interesterification) TAG until a thermodynamic equilibrium is reached (9). The replacement of saturated FA at the *sn*-2 position of butterfat TAG by hypocholesterolemic FA, derived from vegetable oils, is an

\*To whom correspondence should be addressed.

important nutritional consequence of interesterification. A thorough review of the mechanisms of chemical interesterification is presented elsewhere (10).

The primary objective of this study is to produce a butterfat-based spread that is ideally spreadable at both room and refrigerator temperatures, has all the inherent qualities of butter, yet contains "healthy" fatty acids. Therefore, an encompassing study of the macro- and microscopic properties of butterfat, restructured by blending with canola oil and subsequent chemical interesterification, was undertaken. Here, we present the first of a three-part series, in conjunction with a short communication, dealing with melting behavior and TAG modifications produced.

## MATERIALS AND METHODS

**Source materials.** Butterfat was obtained from a local creamery, dried to remove residual moisture with anhydrous sodium sulfate, and filtered through a Whatman #2 filter. Canola oil (Mazola Light brand) was purchased from a local grocery store and used without further treatment. All chemicals were purchased from Fisher Scientific (St. Louis, MO) and used without further purification. The catalyst, sodium methoxide, was stored at room temperature under a nitrogen atmosphere (BOC Gases, Guelph, Ontario, Canada) in a desiccator to avoid decomposition.

**Blend preparation.** Liquefied butterfat and canola oil were mixed in proportions ranging from 100% butterfat to 100% canola oil in 10% increments (w/w). Eleven blends were prepared [butterfat (BF), 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, canola oil], identified by the mass ratio of butterfat to canola oil.

**Chemical interesterification.** Portions (100 g) of the blends were mixed with 0.5% (w/w) sodium methoxide, followed by a thorough nitrogen flush to remove moisture and air. The interesterification reaction was performed at 78–82°C in a stoppered flask in a waterbath with constant agitation. To terminate the reaction, flasks were placed in a lukewarm water bath, followed by addition of excess solid citric acid to neutralize the catalyst. The citric acid and sodium methoxide were removed with warm water washes (3 × 150 mL). Residual water was removed with excess anhydrous sodium sulfate, followed by filtration through a Whatman #2 filter.

To determine the optimal duration of chemical interesterification, samples of 100% butterfat and 100% canola oil were interesterified for 15, 30, 60, 90, and 120 min. The minimal time to provide the maximal change of solid fat content (SFC) was chosen. TAG analyses were performed on both butterfat and canola oil to measure compositional modifications. Non-interesterified is abbreviated to NIE and interesterified to IE.

**Differential scanning calorimetry (DSC).** Calorimetric evaluations of sample melting behavior were performed in a DuPont 1090 DSC. Liquefied samples (9–10 mg) were loaded in aluminium pans and hermetically sealed. All samples were refrigerated at 4°C for 24 h prior to measurement, thus ensuring identical temperature histories. DSC analyses were per-

formed from 0 to 55°C at a scan rate of 5°C/min relative to an empty pan.

Thermograms were analyzed for onset and end of melting, major peak maxima temperatures (°C) and enthalpy of melting (J/g). Data analysis was performed with software provided with the DSC.

**Pulsed nuclear magnetic resonance (pNMR).** SFC was measured by pNMR in a Bruker PC/20 Series NMR Analyzer (Bruker, Milton, Canada) (AOCS official method Cd 16–81). However, the samples were tempered at 25°C instead of 26.7°C. This method consists of heating the fats to 60°C for 30 min, solidifying them at 25 and 0°C for 15 min, tempering at 25°C for 30 min, and then cooling again at 0°C for 15 min. The samples were conditioned for 30 min at the chosen measurement temperature. During this study, the samples were evaluated at 5°C intervals from 5 to 45°C.

**Gas-liquid chromatography (GLC).** TAG composition [carbon number (C)] of blends was determined by GLC with a Shimadzu GC-8A (Tokyo, Japan). One milliliter of liquefied sample was dissolved in 5 mL iso-octane, and 1 µL of dissolved sample was injected into the GLC at 360°C. Runs were performed from 270 to 340°C at 5°C/min and then isothermally for 15 min at 340°C. A 1-m glass column was packed with 3% OV-1 on 80–100 Supelcoport (Supelco, Bellefonte, PA). A reference column was used, and the carrier gas was high-purity nitrogen (BOC Gases). The flame-ionization detector was operated at 360°C.

**Replication and statistical analysis.** Experiments were duplicated, and triplicate analyses were performed on each replicate for pNMR, DSC, and GLC measurements. Statistical analysis was performed by the SAS General Linear Methods procedure (11). Differences were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

**Determination of optimal chemical interesterification duration.** Chemical interesterification is a difficult process to control because it is not well understood. In the literature, most studies dealing with chemical interesterification do not show why a specific reaction time is chosen. Coenen (12) and Weiss *et al.* (13) both stated that the reaction is entropically driven and that, once it has begun, it is a quick process. Other workers (14) have shown that the reaction can continue for many hours. All agree that the reaction is extremely temperature dependent. In our study, we determined the reaction time for maximizing SFC change.

In this study, reaction onset was considered as the appearance of the brown intermediate, commonly associated with the beginning of interesterification (9,12).

Changes in butterfat TAG composition are shown in Figure 1. As interesterification progressed, TAG species  $C_{24}$ – $C_{32}$  and  $C_{44}$ – $C_{48}$  increased at the expense of species  $C_{36}$ – $C_{40}$  and  $C_{52}$ – $C_{54}$ , which decreased.  $C_{50}$  increased by 2% after 30 min, yet decreased by ~5% after 60 min of treatment and then remained constant after 120 min of treatment.  $C_{34}$  decreased by

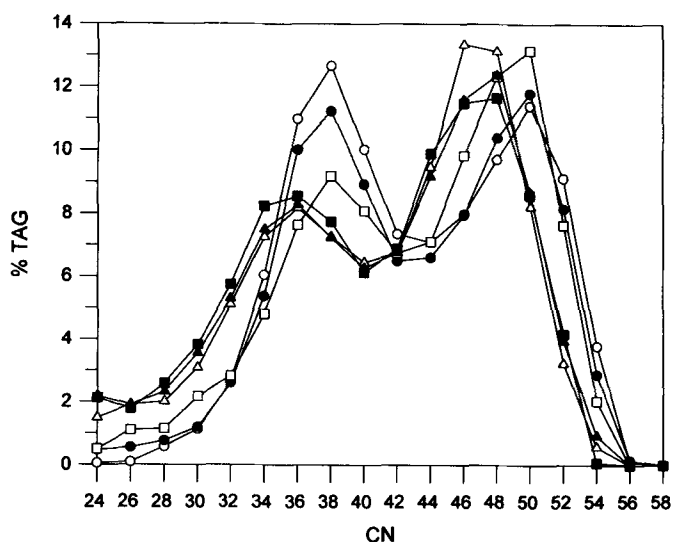


FIG. 1. Effect of chemical interesterification on the relative proportions (w/w) of butterfat triacylglycerols (TAG) as function of TAG species carbon number (CN). Noninteresterified butterfat (○-○), interesterified butterfat—15 min (●-●), 30 min (□-□), 60 min (■-■), 90 min (△-△), and 120 min (▲-▲).

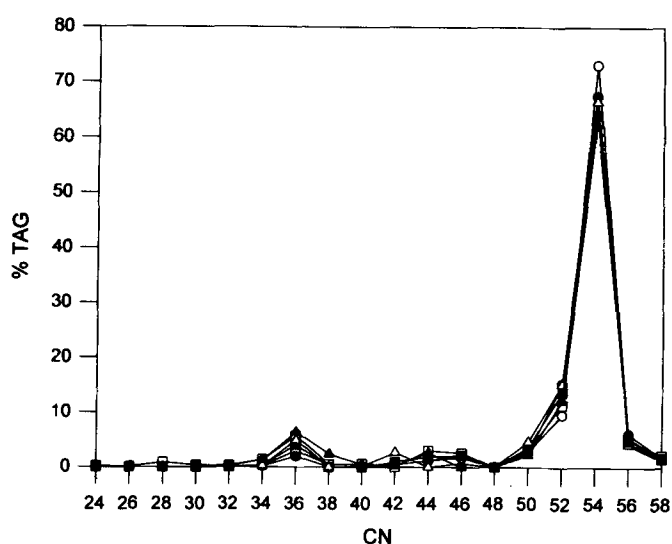


FIG. 2. Effect of chemical interesterification on the relative proportions (w/w) of canola oil TAG as function of TAG species CN. Noninteresterified canola oil (○-○), interesterified canola oil—15 min (●-●), 30 min (□-□), 60 min (■-■), 90 min (△-△), and 120 min (▲-▲). See Figure 1 for abbreviations.

1% after 30 min and then increased by 5% after 120 min. Results showed that interesterification was incomplete after 2 h because noteworthy changes were still observed in the TAG profile between 90 and 120 min, mainly in the  $C_{46}$  species.

Canola oil interesterification (see Fig. 2) generated only small changes in TAG composition compared with butterfat. Because canola oil is composed of ~90% unsaturated  $C_{18}$  FA, there will always be production of  $C_{54}$  (tri- $C_{18}$ ) species, regardless of treatment duration. After 2 h, the TAG profile was not greatly altered.  $C_{54}$  species diminished whereas  $C_{36}$  species increased. Small changes were also observed in the  $C_{52}$  species. The increased proportion of  $C_{36}$  species, most likely  $C_{18}$  diacylglycerols, suggests the presence of unwanted moisture in the system, leading to hydrolysis. The presence of water inactivates the catalyst by converting it to methanol (9).

Physically, the SFC modifications did not change substantially after 30 min of treatment. In Figure 3, the SFC evolution of butterfat is plotted against chemical interesterification duration. After 15 min, the SFC at 5, 10, 15, and 20°C dropped, but increased at 25, 30, and 35°C. After 30 min, the SFC at 15 and 20°C increased 3 and 5%, respectively. SFC values at 5 and 10°C decreased by an additional 1% between 15 and 30 min, whereas SFC at 25, 30, and 35°C increased 2–4%. After 30 min, the SFC variations were small (<1% SFC) except at 10°C where a 2% drop was registered.

Determination of which species are responsible for the observed SFC evolution was difficult to assess. TAG species  $C_{24}$ – $C_{32}$  and  $C_{44}$ – $C_{48}$  increased as did the SFC between 25 and 35°C. TAG species  $C_{36}$ – $C_{40}$  and  $C_{52}$ – $C_{54}$  decreased as did the SFC at 5 and 10°C. The variable SFC pattern at 15 and 20°C may be correlated with the variable patterns of TAG species  $C_{34}$  and  $C_{50}$ .

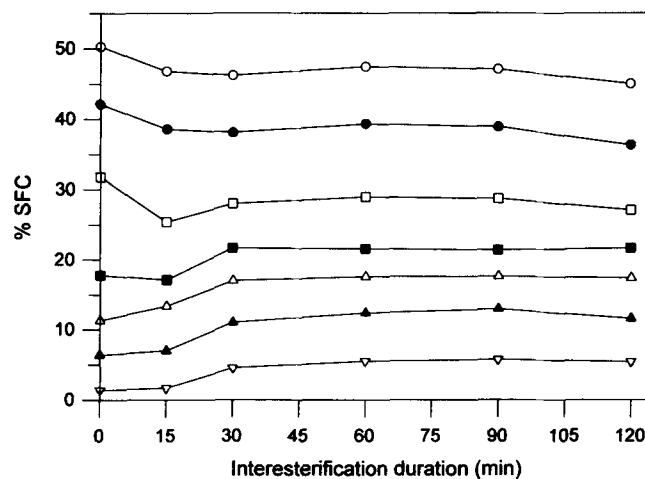


FIG. 3. Solid fat content (SFC) evolution of butterfat as a function of chemical interesterification duration (min); 5°C (○-○), 10°C (●-●), 15°C (□-□), 20°C (■-■), 25°C (△-△), 30°C (▲-▲), and 35°C (▽-▽).

*Blending and chemical interesterification effects on SFC of butterfat blends.* The SFC profiles as functions of temperature for NIE and IE blends of butterfat to 20:80 blends are shown in Figures 4 and 5, respectively.

All blend SFC profiles were significantly different from each other ( $P < 0.0001$ ), and the rate of SFC evolution was dependent on both temperature and the proportion of butterfat in the blend ( $P < 0.0001$ ).

A nonlinear profile was evident for all blends. NIEBF and the NIE90:10 and NIE80:20 blends had similar melting profiles. The NIEBF blend had an SFC of 49% at 5°C, which progressed downward nonlinearly until no solid fat was present at 40°C. The NIE90:10 and NIE80:20 blends were also completely melted at 40°C, but they had lower SFC at 5°C.

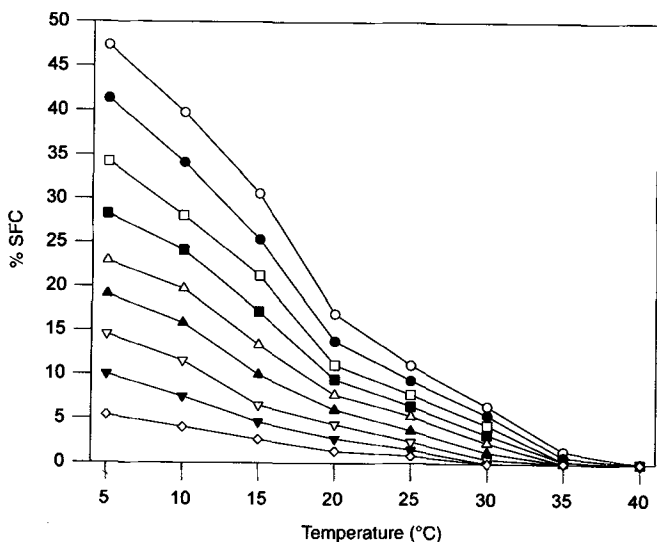


FIG. 4. SFC evolution of noninteresterified butterfat-canola oil blends as a function of temperature ( $^{\circ}\text{C}$ ); butterfat (○-○), 90:10 (●-●), 80:20 (□-□), 70:30 (■-■), 60:40 (△-△), 50:50 (▲-▲), 40:60 (▽-▽), 30:70 (▼-▼), and 20:80 (◇-◇). See Figure 3 for abbreviation.

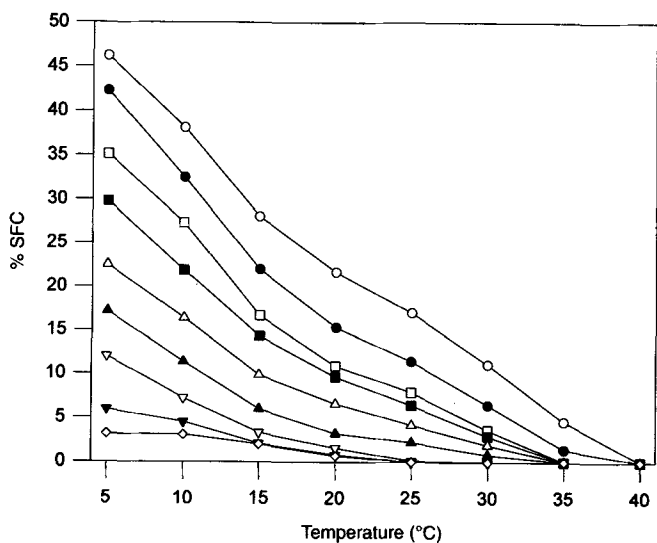


FIG. 5. SFC evolution of interesterified butterfat-canola oil blends as a function of temperature ( $^{\circ}\text{C}$ ); butterfat (○-○), 90:10 (●-●), 80:20 (□-□), 70:30 (■-■), 60:40 (△-△), 50:50 (▲-▲), 40:60 (▽-▽), 30:70 (▼-▼), and 20:80 (◇-◇). See Figure 3 for abbreviation.

Other groups of blends with similar profiles included the NIE70:30 and NIE60:40 blends, the NIE50:50 and NIE40:60 blends, and the NIE30:70 and NIE20:80 blends. The largest decline in SFC occurred from 15 to 20 $^{\circ}\text{C}$ , due to the large proportion of TAG that liquefy and solubilize in this range. However, as the proportion of oil was increased, this sharp drop became less pronounced. For the NIE50:50 and NIE40:60 blends, the rate of melting was slower at 15–20 $^{\circ}\text{C}$  than between 10 and 15 $^{\circ}\text{C}$ . The NIE60:40 and NIE70:30 blends have similar melting behavior between 10 and 20 $^{\circ}\text{C}$ .

Progressively lower concentrations of butterfat led to a reversal of melting behavior between 10 and 20 $^{\circ}\text{C}$ .

The SFC profiles of IE blends differed from those of the NIE blends (Fig. 5). The most striking feature was the disappearance of the sharp SFC drop in the 15–20 $^{\circ}\text{C}$  which shifted to 10–15 $^{\circ}\text{C}$  for most of the IE blends.

The profile of the IE blends mimicked that of IEBF but at lower SFC values. As with simple blending, the complexity of the profile diminished as the percentage of oil increased and became quasi-linear for the IE20:80 and IE30:70 blends. The increased linearity was due to the greater variation of TAG that resulted from interesterification (15).

The proportion of butterfat and interesterification (in the 5–20 $^{\circ}\text{C}$  range only) had a significant effect on SFC ( $P < 0.0001$ ). SFC was not significantly affected by chemical interesterification at 25 and 30 $^{\circ}\text{C}$  ( $P > 0.05$ ).

The contour profile (Fig. 6) displays the effect of chemical interesterification on blend SFC. No SFC changes greater than  $\pm 6\%$  resulted from this treatment. The largest SFC increase was produced by interesterification of butterfat at 25 $^{\circ}\text{C}$ , while SFC “valleys” were present for the 80:20 blend at 15 $^{\circ}\text{C}$ , and the 40:60 and 50:50 blends at 10 $^{\circ}\text{C}$ . The most dramatic transition in SFC as a function of temperature occurred with 100% butterfat, judging from the proximity of the contour lines. Increases in SFC occurred only for the butterfat and the 90:10 blend. Obviously, no large change in SFC occurred at higher temperatures. The SFC drops upon interesterification are due to the replacement of saturated FA with unsaturated fatty acids in the butterfat TAG, which contains many di- and trisaturates. Due to the presence of double bonds, unsaturated fatty acids have lower melting points.

A look at the isosolids diagram (Fig. 7) reveals continuous solid solutions for both NIE and IE blends. Isosolid lines represent the temperature at constant SFC for various blends of

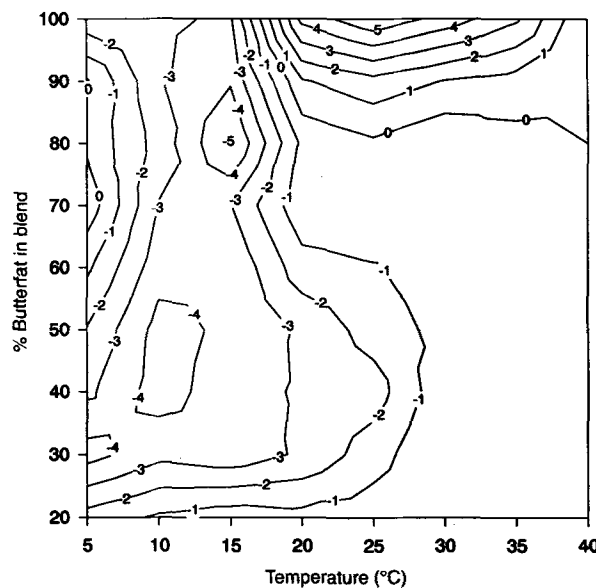


FIG. 6. Contour profile of the effect of chemical interesterification on the solid fat content of butterfat-canola oil blends. Each line represents a 1% change in solid fat content.

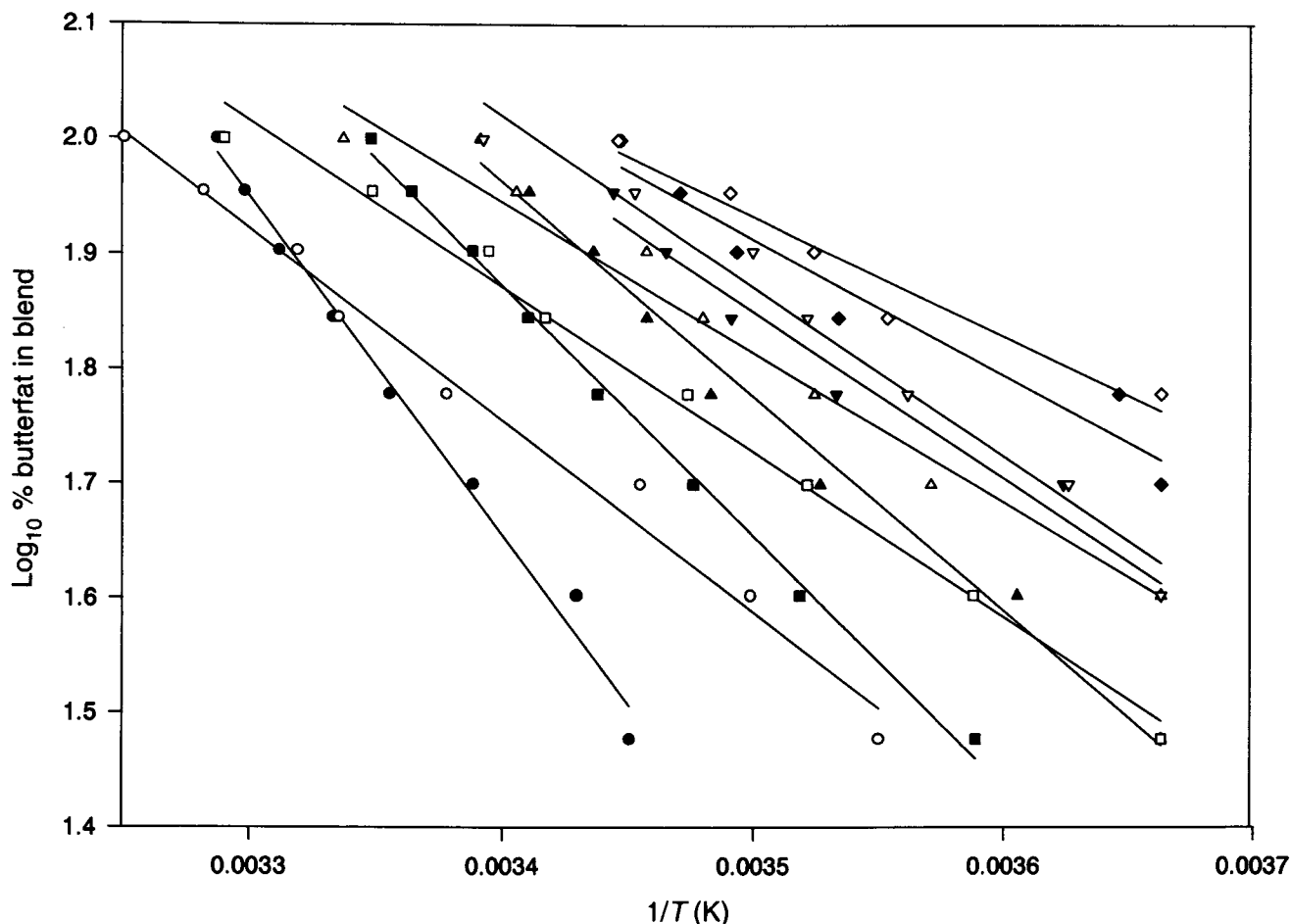


FIG. 7. Isosolid lines as a function of  $\log_{10}$  % butterfat in each blend. 5% Isoline-noninteresterified (NIE) (●-●), 5% isolate-interesterified (IE) (●-●); 10% isolate-NIE (□-□), 10% isolate-IE (■-■); 15% isolate-NIE (△-△), 15% isolate-IE (▲-▲); 20% isolate-NIE (▽-▽), 20% isolate-IE (▼-▼); 25% isolate-NIE (◇-◇), and 25% isolate-IE (◆-◆).

fats. For all linear regressions, the correlation coefficients ( $r$ ) were greater than 0.95. Trends in isolines indicate the dilution effect generated by blending and chemical interesterification. Quite visible is the greater dilution effect for NIE blends than for IE blends, due to the greater intersolubility of "butterfat" TAG after interesterification. A crossover occurred between the 80:20 and 70:30 blends for the 5, 10, and 15% isolines. Large differences in slope exist between these sets of linear regressions, but the difference between the slopes diminished with increasing iso-SFC value. By 20% SFC, the isolines were parallel. No crossover was observed at the 25% SFC lines; however, the lines were not parallel.

**DSC analysis of restructured butterfat.** DSC thermograms, which revealed transition temperatures and heats of fusion, provided complementary results to the pNMR data. Thermograms, representative of NIE and IE butterfat and the 90:10 and 50:50 blends, are shown in Figure 8, with the shaded area representing the IE blends. As with pNMR, interesterification produced noteworthy changes in melting profile, whereas blending alone resulted in a dilution effect.

The melting thermograms for butterfat were similar to pre-

viously published results (16,17). Because butterfat is a mixture of many compounds, it exhibited a broad melting range that was composed of the low-melting TAG (LMTAG), the middle-melting TAG (MMTAG), and the high-melting TAG (HMTAG). Smaller peaks were also resolved in the thermogram, suggesting possible polymorphic subforms due to lack of thermodynamic equilibrium. Interesterification of the butterfat caused HMTAG to increase at the expense of the MMTAG. The endotherms of LMTAG diminished after interesterification. The butterfat thermogram shows that the LMTAG end of melt was at  $\sim 19^{\circ}\text{C}$ , the MMTAG fraction at  $\sim 35^{\circ}\text{C}$ , and the HMTAG fraction at  $\sim 48^{\circ}\text{C}$ . Thus, at refrigerator temperature, butterfat was composed of all three fractions, whereas at room temperature, the solid butterfat contained mainly the MMTAG and HMTAG fractions. The same analysis could apply to IEBF.

Addition of 10% canola oil (NIE90:10) produced a dilution effect of the profile of the NIEBF (Fig. 8B). Interesterification, however, created a much altered thermogram. The MMTAG fraction diminished, while the HMTAG fraction shifted a few degrees lower and the LMTAG became more pronounced.

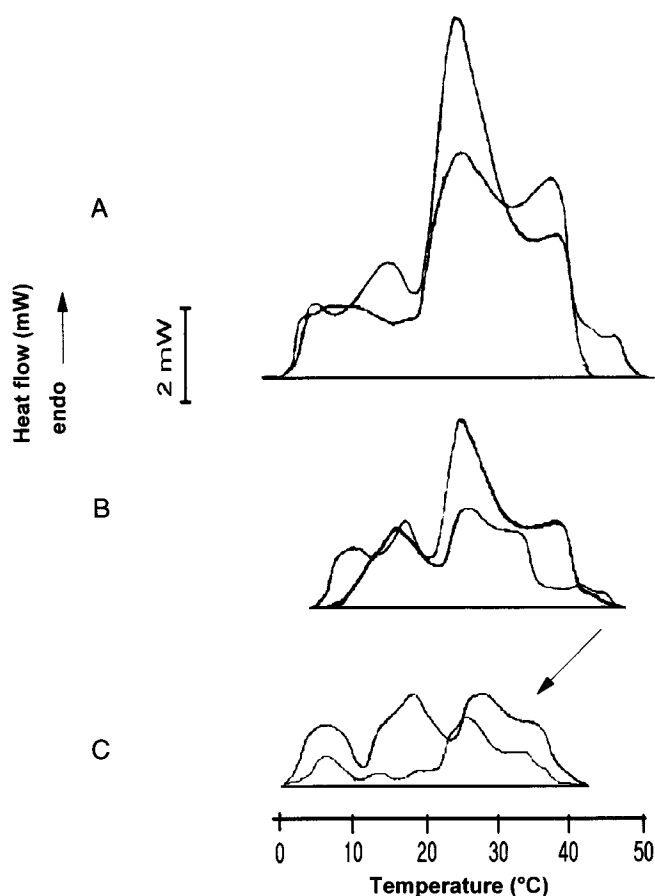


FIG. 8. Differential scanning calorimetry melting thermograms of non-interesterified and interesterified (IE) blends; A, butterfat; B, 90:10; and C, 50:50. The shaded area represents the IE blends.

Incorporation of 50% canola oil led to peak separation (Fig. 8C) of the remaining solid fat, while interesterification dramatically altered the melting profile as the MMTAG fraction disappeared.

Onset of melting, peak maxima for the three main peaks, and end of melting temperatures for the DSC thermograms as a function of butterfat proportion are shown in Figure 9. The peak maxima did not change much as the canola oil content increased. Onset of melting temperatures for NIE blends was 1.7°C for NIEBF and then between 4.3 and 4.8°C for all other blends (NIE90:10 to NIE30:70). NIEBF onset temperature was significantly different ( $P < 0.05$ ) from the onset temperature of all other blend. For IE blends, the onset temperature was more random. IEBF, IE90:10, and IE70:30 blends had onset temperatures between 2 and 3°C, while the onset temperature of the IE80:20 blend was 3.9°C. The IE60:40, IE50:50, and IE30:70 blends had an onset temperature of 4.2°C, while the IE40:60 blend began to melt at 5.6°C. Onset temperatures should probably have been lower for IE blends because the integrity of the butterfat TAG was altered and they were more unsaturated.

The profile of onset temperatures for NIE blends was not expected to change dramatically as the percentage of oil in-

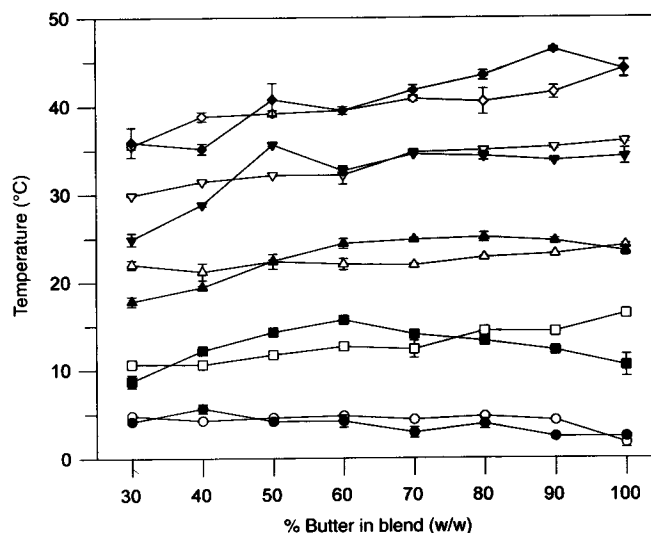


FIG. 9. Transition temperatures (onset of melt, peak maxima, and end of melt) for noninteresterified (NIE) and interesterified (IE) blends of butterfat and canola oil; onset—NIE blends (○-○), IE blends (●-●); peak 1 maxima—NIE blends (□-□), IE blends (■-■); peak 2 maxima—NIE blends (△-△), IE blends (▲-▲); peak 3 maxima—NIE blends (▽-▽), IE blends (▼-▼), and end of melting—NIE blends (◇-◇) IE blends (◆-◆).

creased. The enthalpy should drop linearly while the temperatures of onset and end of melting do not change. However, with the incorporation of 10% or more canola oil, the onset temperature increased by ~2°C. This means that there were less TAG crystallizing at low temperature, possibly indicative of increased solubility of the LMTAG in the canola oil. This increased solubility was constant for between 10 and 70% canola oil in the blend because there was no subsequent increase in onset temperature. For the IE blends, a general upward trend was observed. This was expected because more intermediate TAG were produced. Similar TAG are more likely to dissolve in one another ("like dissolves like"). Statistically, butterfat content had a significant effect ( $P < 0.0001$ ) on onset of melting, whereas interesterification did not ( $P > 0.2$ ).

The same analysis can be applied to the end of melt temperatures. For NIEBF, end of melting was observed at 44.2°C; addition of 10% canola oil resulted in a 2.7°C drop to 41.5°C. Between NIE90:10 and NIE40:60, the end of melt temperature dropped only 2.6°C. Finally, the NIE30:70 blend finished melting at 35.5°C, 3.3°C lower than the 40:60 blend. There appeared to be three regions of miscibility—NIEBF, NIE90:10–NIE40:60, and NIE30:70. The miscibility was dependent on the proportion of butterfat TAG. Butterfat concentration had a significant effect ( $P < 0.0001$ ) on end of melt temperatures, whereas interesterification did not ( $P > 0.35$ ).

A comparison of all peak maxima trends between the IE and NIE blends revealed similar results. IEBF had lower maxima than NIEBF. With gradual incorporation of oil, an inversion occurred, and the IE blends had higher maxima than NIE blends. This inversion occurred at different butterfat–canola

oil proportions, depending on the peak: peak 1 between 80:20 and 70:30; peak 2 between butterfat and 90:10; and peak 3 between 70:30 and 60:40. Another crossover occurred at higher canola oil concentrations: peak 1 between 60:40 and 30:70; peak 2 at 50:50; and peak 3 between 50:50 and 40:60.

As shown in Figure 8, peak 2 corresponded to the MMTAG fraction, the largest fraction in butterfat. This fraction was least affected in terms of peak maxima by the addition of canola oil because the oil seemed to partition primarily to the butterfat LMTAG and HMTAG fractions.

Heat of fusion (enthalpy) was proportional to sample SFC at a specific temperature (Fig. 10). As expected, the enthalpy dropped as canola oil was added. Canola oil does not crystallize at refrigerator temperatures and cannot be directly implicated in the crystallization of the blends *per se*. However, it was involved in intimate mingling with the butterfat TAG. The enthalpy for NIE blends was much higher than for IE blends, and the drop in enthalpy observed with addition of oil was not linear. For blends with 70% or more butterfat, the enthalpy diminished slowly in an almost linear fashion. NIEBF to NIE70:30 enthalpies were not significantly different from each other ( $P > 0.05$ ). With 50% or less butterfat, the enthalpy drop was much sharper. These results suggest that the butterfat TAG matrix could harbor a large amount of liquid oil and retain a structured network. The NIE60:40 region may represent an important region in the crystallization pattern of these blends.

The drop in enthalpy of the IE blends was not linear as oil content increased, and there was no obvious break point as with NIE blends, due to the randomization of the TAG structure. Fewer trisaturates did not permit the formation of a strong network, hence less heat was required to melt the crystal network.

**TAG modifications and effects on enthalpy of melting.** In any fat, there is a direct relationship between the TAG composition and physical properties. The types of FA dictate the melting behavior of a TAG. Also important is the FA positioning along the glycerol backbone, which affects structure

and hence melting. TAG with a more asymmetrical distribution tend to have lower melting points. Given butterfat's complex composition, determination of the melting pattern is complicated.

The proportion of butterfat had a significant effect ( $P < 0.05$ ) on TAG composition, except for carbon species  $C_{42}$ . Chemical interesterification produced significant changes in TAG composition for most carbon species in most samples, except species  $C_{32}$ ,  $C_{34}$ ,  $C_{38}$ , and  $C_{42}$  ( $P > 0.05$ ). For carbon species  $C_{24}$  to  $C_{30}$  and  $C_{58}$ , no changes greater than 1.5% were observed.

Butterfat TAG were divided into two groups (Fig. 1), the first composed of carbon species  $C_{26}$ – $C_{40}$  and the second composed of  $C_{44}$ – $C_{54}$ . The predominant TAG in butterfat were  $C_{36}$  (11.0%),  $C_{38}$  (12.7%),  $C_{40}$  (10%),  $C_{48}$  (9.7%),  $C_{50}$  (11.4%), and  $C_{52}$  (9.1%). According to Timms (17), the LMTAG fraction is composed of TAG that contain one long-chain saturated acid with two short-chain or *cis*-unsaturated acids. The MMTAG fraction TAG contain two long-chain saturated acids plus one short-chain or *cis*-unsaturated acid. Finally, the HMTAG fraction contains only long-chain saturated acids.

This great variety in TAG was not found in canola oil, which was predominantly composed of  $C_{54}$  (73.2%) and  $C_{56}$  (9.6%) (Fig. 2). According to Sebedio and Ackman (18), canola oil contains 60%  $C_{18:1}$ , 20%  $C_{18:2}$ , and 12%  $C_{18:3}$ .

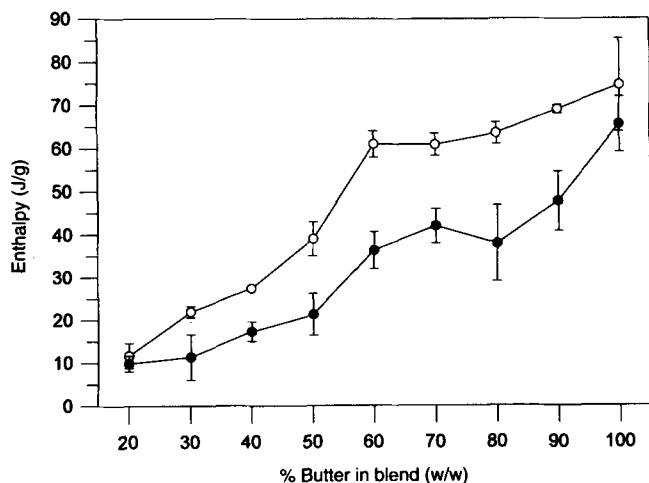
Small TAG distribution changes occurred with addition of 10% canola oil (Fig. 11A), which correlated to the small drop in enthalpy from 100% butterfat (Fig. 10). Interesterification led to a substantial drop (31%) in the enthalpy of IE90:10.

Gradual TAG modifications were observed for the 80:20 to 60:40 blends (Fig. 11, B–D). The  $C_{34}$ – $C_{40}$  and  $C_{54}$  species diminished, whereas the  $C_{46}$ – $C_{52}$  species increased after interesterification. These gradual TAG changes accounted for the steady changes in enthalpy and melting.

The notable change in enthalpy for the 50:50 blend (Fig. 11E) cannot be readily explained by the TAG behavior that underwent a gradual change from NIE60:40 to NIE50:50. Perhaps there is a limit of saturated TAG required to hold the crystal network together. The most notable changes occurred in the  $C_{50}$ – $C_{54}$  species.

For all other blends (Fig. 11, F–I), TAG modifications were gradual, except for the 40:60 and 20:80 blends, which underwent substantial changes, mainly in the  $C_{54}$  species. With the loss of  $C_{54}$ , there were large increases in the  $C_{48}$ – $C_{52}$  species. This exchange was not reflected in the enthalpy changes, which had a constant rate of decline for these blends.

**Thermodynamic considerations.** With the Hildebrand solubility law, predicting the melting point of a higher-melting lipid component in a lower-melting component is possible (19). This equation is designed for ideal mixtures (20). In the liquid state, above the temperature at which no crystal structure is present (4), all TAG should be mutually soluble. As the temperature is decreased from the melt and the lipid is supercooled, the solubility of the crystallizing TAG becomes negligible (21). The following equation permits an approximation of the melting of the butterfat component in canola oil:



**FIG. 10.** Enthalpy of melting (J/g) of interesterified (IE) and noninteresterified (NIE) butterfat–canola oil blends as a function of % w/w butterfat in the blend; NIE blends (○-○) and IE blends (●-●).

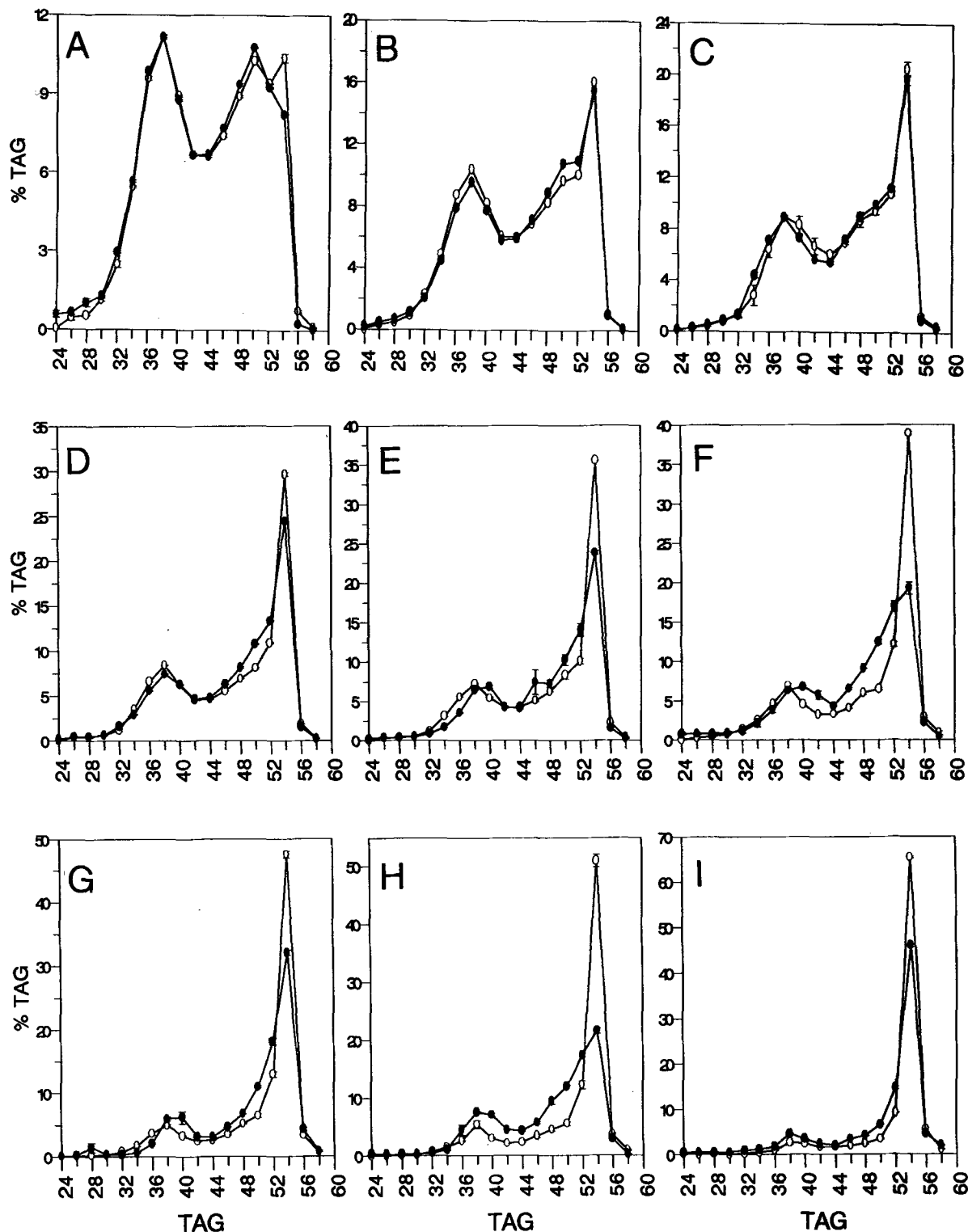


FIG. 11. Relative triacylglycerol (TAG) proportion (% w/w) evolution as a function of TAG species carbon number for noninteresterified (NIE) and interesterified (IE) butterfat-canola oil blends; NIE blends (○-○) and IE blends (●-●); A, 90:10; B, 80:20; C, 70:30; D, 60:40; E, 50:50; F, 40:60; G, 30:70; H, 20:80; I, 10:90.

$$\ln x = \frac{\Delta H_f}{R} \left( \frac{1}{T_m} - \frac{1}{T} \right)$$

[1]

where  $x$  = mole fraction of the high-melting lipid [butterfat molecular weight -731 (22), canola oil molecular weight -876 (22)],

$\Delta H$  = the enthalpy of HMTAG (J/mol),  $R$  = universal gas constant (8.314 J/mol K),  $T_m$  = melting temperature (K) of HML, and  $T$  = melting temperature (K) of blend.

Temperature deviations from ideal solubility for butterfat to 20:80 blends before and after interesterification are shown in Table 1. The solubility of butterfat became progressively



**TABLE 1**  
Temperature Deviations ( $\pm$ SE) from Ideal Solubility for Butterfat to 20:80 Blends Before and After Interesterification as Evaluated by Hildebrand Solubility Law for Ideal Mixtures

% Butter in blend	Deviation ( $^{\circ}$ C) from ideal solubility	
	Noninteresterified	Interesterified
90	$-1.29 \pm 0.01$	$-1.51 \pm 0$
80	$-2.84 \pm 0.03$	$-3.30 \pm 0$
70	$-4.48 \pm 0.01$	$-5.12 \pm 0.03$
60	$-6.49 \pm 0.03$	$-7.37 \pm 0.02$
50	$-8.80 \pm 0.02$	$-10.09 \pm 0.11$
40	$-11.60 \pm 0.04$	$-12.84 \pm 0.05$
30	$-14.95 \pm 0.03$	$-16.96 \pm 0.18$

higher than predicted by the Hildebrand equation, for both IE and NIE samples, with an increase in the proportion of canola oil. As expected, the deviation was higher for IE samples because the butterfat component (HMTAG) was altered through interesterification, leading to a greater variety in TAG.

Statistically, both interesterification and blending had significant effects ( $P < 0.0001$ ) on deviation from ideal solubility. All deviations from ideal solubility for all blends were significantly different from one another ( $P < 0.05$ ).

It is the nature of any system to try to be in its least energetic state. The change in free energy determines the tendency of a reaction to occur. Bailey (23) described entropy as freedom or randomness in a fat system. In the solid state, order predominates. Solid-liquid transformations result in increased entropy due to increased atomic and electronic motion. Heating of fat samples by DSC similarly results in increased structural disorder. However, because the temperature does not increase during the solid to liquid transformation, the energy released is strictly entropic in nature. TAG encased in a crystal matrix with reduced molecular motility become free to move upon liquefaction, resulting in higher entropy. According to Hildebrand and Scott (19), the following equation describes entropy at the melting point:

$$\Delta S_m = \frac{\Delta H_m}{T_m} \quad [2]$$

where  $\Delta S_m$  = change in entropy (kJ/mol K),  $T_m$  = melting temperature (K), and  $\Delta H_m$  = enthalpy (kJ/mol).

Table 2 lists the entropy of melting of butterfat to 30:70 blends. NIEBF had the highest change in entropy (most order lost during melting). As gradual amounts of oil were added to the blends,  $\Delta S_m$  dropped nonlinearly. At 50% butterfat, there was a large drop in  $\Delta S_m$ , indicative of a much less ordered system. Depending on the blend, the change in entropy was 1–2 times smaller for the IE blends than for the NIE blends. Greater changes in entropy were obtained for the NIE blends due to their greater initial order.

In summary, this study has shown that blending and chemical interesterification significantly modified the physical and chemical properties of butterfat. The most striking feature was that constant increments of canola oil, followed by chemical in-

**TABLE 2**  
Entropy of Melting for Butterfat to 20:80 Blends Before and After Interesterification

% Butter in blend	Entropy of melting <sup>a</sup> (kJ/mol K)	
	Noninteresterified	Interesterified
100	0.1720	0.1511
90	0.1605	0.1092
80	0.1480	0.0874
70	0.1419	0.0973
60	0.1427	0.0849
50	0.0912	0.0496
40	0.0642	0.0410
30	0.0519	0.0268

<sup>a</sup>SE was  $< 10^{-4}$  kJ/mol K.

teresterification, do not produce constant and linear changes in melting and TAG behavior. Instead, the properties of the blends were unpredictable and depended heavily on the proportions of the "parent" lipids.

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