

## Comment on the Use of Direct Pulsed Nuclear Magnetic Resonance Solid Fat Content Measurements in Phase Behavior Studies of Lipid Mixtures

Sir:

Pulsed nuclear magnetic resonance (pNMR) can be used to determine the solid fat content (SFC) of lipid mixtures in phase behavior studies. Determined by the direct method, and using standards calibrated in mass percentage of solids, the measured SFC is a mass fraction quantity. Further assumptions made are that the hydrogen nuclei contents and relaxation properties ( $T_2$  or spin-spin relaxation time) of the lipid and the standards are similar.

For phase behavior studies, molar fractions, rather than mass fractions, of solute in solvent are required. When all the molecules in a sample blend are of similar molecular weight, the standard mass-SFC determination provides a good approximation of the molar-SFC. However, when the molecular weights of the blended solute and solvent differ, mass-SFC can differ greatly from molar-SFC. For this reason, caution should be exercised when interpreting experimental results of the phase behavior of lipid mixtures using standard mass-SFC measurements.

In a recent study by Wright *et al.* (1) on the effects of solvents on the crystallization behavior of milk fat fractions, we were reminded of this need for caution. The high-melting fraction (HMF) and middle-melting fraction (MMF) of milk fat were blended with milk fat's low-melting fraction (LMF), canola oil, ethyl acetate, and hexane in 0, 10, 25, 50, 75, and 100 mol% ratios. SFC were measured by pNMR with a Bruker PC20 Series NMR analyzer (Bruker, Milton, Ontario, Canada) as described previously (1). Blends of HMF and tricaprylin (100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 mol%) were later prepared to further investigate possible discrepancies between mass-SFC and molar-SFC for blends of triglycerides with more dissimilar molecular weights than the milk fat fractions. Mass-SFC measurements were converted to molar-SFCs using Equation 1.

$$\text{SFC(mol\%)} = \frac{\text{SFC(mass\%)} \cdot (M_A^T + M_B^T)}{M_A^T + M_B^T \cdot \frac{\text{MW}_A}{\text{MW}_B}} \quad [1]$$

$M^T$  corresponds to the total mass of component A or B, while MW corresponds to the molecular weight (g/mol) of component A or B. Knowledge of blend ratios, total sample weights, and known molecular weights of the fat and solvent (HMF,

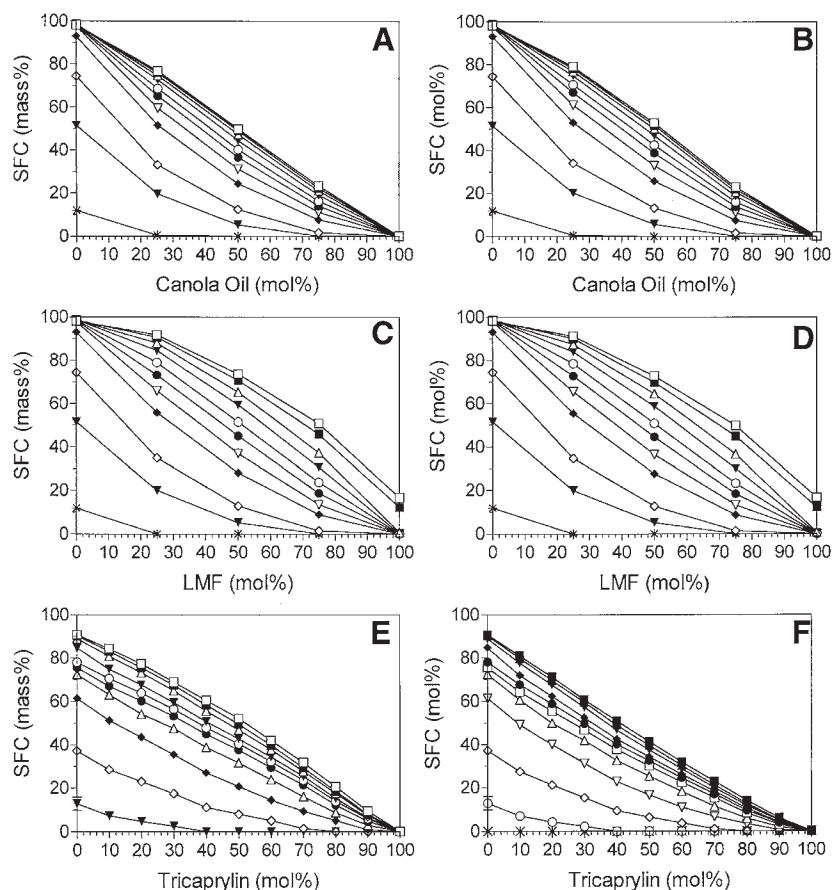
787.96; MMF, 755.74; LMF, 774.68; canola oil, 878.86; ethyl acetate, 88.11; hexane, 86.18; and tricaprylin, 470.03 g/mol) was required for this purpose. The molecular weights of the milk fat fractions were calculated using fatty acid composition data.

For example, a 2.0 g, 20% SFC blend contains 0.4 g of solid and 1.6 g of liquid. Assuming that the added solvent does not crystallize, knowledge of the mass of noncrystallizing solvent will then allow for the calculation of the molar-SFC parameter. The ratio of the calculated molar-SFC to the original mass-SFC provides a conversion factor which can be applied to all other mass-SFC readings, at different temperatures, for that particular blend.

Equation 1 is valid in cases where the solvent and solute triacylglycerols do not co-crystallize. In cases where co-crystallization occurs, but the molecular weights of the component triacylglycerols are similar, the equation will still be approximately correct. Attempts were also made to correct for differences in the number of protons per molecule and the dependence of the relaxation behavior ( $T_2$ ) on the physical state of the molecules (solid/liquid ratio). However, as this proved to be more complicated than expected, these attempts were unsuccessful. The presence of a semisolid state in milk fat, as demonstrated by Le Botlan *et al.* (2), could also influence the pNMR results. Despite these limitations, Equation 1 provides a reasonable conversion to compare mass and molar SFC.

The same trends were observed for HMF and MMF, but for the sake of brevity, only the case for HMF will be discussed. In Figure 1 mass-SFC and molar-SFC for blends of HMF-canola oil (A and B), HMF-LMF (C and D), and HMF-tricaprylin (E and F) are plotted as a function of increasing molal solvent fraction (%). Figure 2 shows the same comparison for HMF-ethyl acetate (A and B) and HMF-hexane (C and D). These graphs reveal three different phase behaviors: dilution, solubilization, and complex formation. A linear decrease in the curve represents a dilution effect. Dilution is a colligative property, depending only on the number of molecules present in a system. At increasing solvent concentrations, there are fewer molecules of triglycerides available to crystallize, hence less solid is formed. Solubilization of solids in the liquid solvent is demonstrated in these diagrams by a nonlinear curve, where the molar fraction of solids is lower than expected solely on the basis of dilution. Interactions with the solvent cause the solubilization of would-be-solid triglycerides, resulting in less solid being formed. Lastly, the amount of solid can be higher

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**FIG. 1.** Solid fat content (SFC) (mass and molar%) of blends of milk fat's high-melting fraction with canola oil (A and B), milk fat's low melting fraction (LMF) (C and D), and tricapyrylin (E and F) at different solvent molal fractions (%) at various temperatures (-□- 0°C, -■- 5°C, -△- 10°C, -▽- 15°C, -○- 20°C, -●- 25°C, -▽- 30°C, -◆- 35°C, -◇- 40°C, -▼- 45°C, and -\* 50°C).

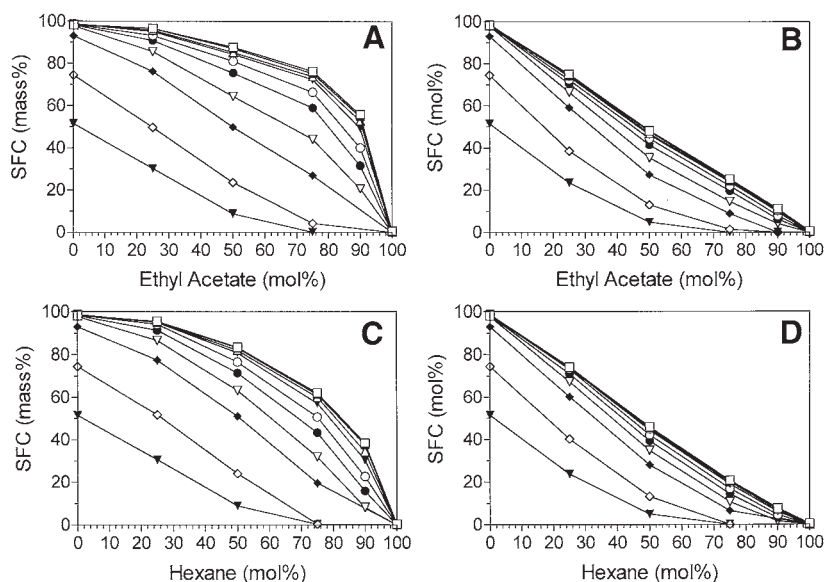
than expected from dilution effects when complexes form between triglycerides of the solute and solvent. The crystallizing solute triglycerides pull some of the would-be-liquid solvent triglycerides into the solid phase.

Figures 1 and 2 demonstrate that very different behaviors would be predicted if mass-SFC rather than molar-SFC were used. HMF, canola oil, and LMF have very similar molecular weights, hence mass-SFC is nearly equivalent to molar-SFC for blends of HMF with these liquid oils (Fig. 1A–D). At low temperatures canola oil dilutes HMF, with more solubilization occurring at higher temperatures (Fig. 1A and B). Both mass-SFC and molar-SFC plots show complex formation between HMF and LMF at low temperatures (0–15°C) and solubilization of HMF in LMF at higher temperatures (20°C and above) (Fig. 1C,D). When different triacylglycerols or fats are blended together, differences may be significant. This is demonstrated in Figure 1E,F where HMF, with molecular weight of 787.96 g/mol, is blended with tricapyrylin, a medium-chain triacylglycerol with molecular weight of 470.03 g/mol. When mass-SFC is used, slight complex formation is indicated at low temperatures because the fraction of solids present is higher than expected due to a dilution effect (Fig. 1E). On the other hand,

only dilution and solubilization effects are evident when molar-SFC is used (Fig. 1F). Here the difference of about 300 g/mol between the molecular weights of HMF and tricapyrylin is large enough to suggest very different phase behaviors in the mass- and molar-SFC plots.

When differences in molecular weights between compounds present in a mixture are more extreme, however, very different conclusions are drawn from the mass-SFC and molar-SFC plots. For example, in Figure 2 the roughly 10-fold difference in molecular weights between HMF and the organic solvents resulted in significant conversion factors, particularly at low solute concentrations, where the value of molar-SFC was less than half of the mass-SFC value. Figures 2A and 2B erroneously suggest that solid complex formation occurs between HMF and the solvents at low temperatures. However, when the molar-SFC are used, only dilution and solubilization effects are observed (Fig. 2B,D). Hexane solubilized more HMF than ethyl acetate (Fig. 2C,D). This clearly demonstrates the potential for misinterpreting phase behavior results when mass-SFC measurements are employed.

Mass-SFC is essentially equivalent to molar-SFC when fats of similar molecular weights are blended so the problems of



**FIG. 2.** SFC (mass and molar%) of blends of milk fat's high-melting fraction with ethyl acetate (A and B) and hexane (C and D) at different solvent molal fractions (%) at various temperatures (-□- 0°C, -■- 5°C, -△- 10°C, -▼- 15°C, -○- 20°C, -●- 25°C, -▽- 30°C, -◆- 35°C, -◇- 40°C, -▼-45°C, and -\* 50°C). For abbreviation see Figure 1.

using standard mass-SFC measurements in phase behavior studies of lipid mixtures are minimized. Certainly, however, there are many instances where this is not the case and caution is required if accurate interpretations are to be made on the phase behavior of lipid mixtures.

## REFERENCES

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