Chemical and Thermal Characteristics of Milk-Fat Fractions Isolated by a Melt Crystallization

P.S. Dimick*, S. Yella Reddy[1], and G.R. Ziegler

Department of Food Science, The Pennsylvania State University, University Park, Pennsylvania 16802

ABSTRACT: Anhydrous milk fat (AMF) was fractionated by a two-stage dry fractionation process to produce three fractions: high melting (HMF), middle melting (MMF), and low melting (LMF). The HMF (m.p. 42°C) exhibited a broad melting range similar to a plastic fat. The MMF (m.p. 33°C) resembled the original AMF (m.p. 31°C), but with slightly higher solid fat content. The LMF (m.p. 16°C) was liquid at ambient temperature. Differences in the thermal properties of these fractions were attributed to the triacylglycerols (TAG) and their fatty acid composition. Saturated TAG with carbon numbers of 36–54 were concentrated in the HMF; whereas unsaturated TAG of carbon number 36–54 predominated in the LMF. Likewise, the long-chain saturated fatty acids were significantly higher and the long-chain unsaturated fatty acids were significantly lower in the HMF fraction. Binary blends of milk-fat fractions with a range of melting profiles were produced by mixing HMF with AMF, MMF, or LMF. Laboratory-prepared fractions were similar to commercially available fractions.

JAOCS 73, 1647–1652 (1996).

KEY WORDS: Dry fractionation, fatty acid, milk fat, thermal properties, and triacylglycerol composition.

Anhydrous milk fat (AMF) is a complex mixture of triacylglycerols (TAG), comprised of at least 60 different fatty acids, with unique chemical and thermal properties. In its original form, milk fat lacks certain physical and functional properties, which limits its utilization (1–5). For example, milk fat lacks the appropriate plasticity and hardness for use in pastries, and its melt profile results in poor spreadability and promotes softening in chocolate (5). The functionality of milk fat may be enhanced by separating AMF into various fractions that have unique functional properties. Proposed food applications for AMF fractions include spreads and cold-spreadable butters, puff pastries, biscuits, shortbreads, cakes, pourable frying oils, cocoa butter replacement in chocolate and confectionery products, and in ice cream and whipped creams (1–5). The high-melting fractions have been shown clearly to inhibit fat bloom in dark and milk chocolate more effectively than original AMF (6,7).

The AMF can be fractionated by a wide variety of techniques, resulting in a range of melting points and different product characteristics. Fractionation of AMF at various temperatures with and without solvents, and with detergents and supercritical carbon dioxide has been reported earlier (3,8–11). Use of solvents or surfactants is more efficient in the separation of TAG, but solvent and detergent removal becomes costly for use in food products (12). Melt or dry fractionation is more common and safe for products used for food applications. Rate of cooling and crystallization temperatures greatly influence the physical characteristics and ease of filtration; large crystals, obtained by slow cooling rates and slow agitation, yield more efficient separation of stearin from olein (13,14).

Milk fat exists primarily in three polymorphic forms: α, β', and β (15). Rapid cooling of milk fat leads to crystals of the unstable α form. Upon holding, transition to more stable crystal forms occurs but, even after long storage periods, α and β' crystals may be present together. The formation of mixed polymorphic crystals in milk fat has been reported. If cooling occurs rapidly, a large number of low-melting TAG are trapped in the crystal lattice formed by the high-melting TAG. Therefore, milk fat that has been cooled rapidly contains more liquid fat at a given temperature than milk fat that has been cooled slowly or stepwise with suitable holding times to promote partitioning of the liquid and solid fractions. Differential thermal analysis has demonstrated that, during rapid cooling of milk fat, uniform crystal formation takes place, but slow or stepwise cooling promotes fractionation of the fat (16). Belousov and Vergelesov (16) found that, during slow cooling of milk fat, crystallization by aggregate carbons takes place due to the complexity and melting range of the TAG and thus, milk fat crystallizes as mixed crystals that display multiple endotherms. This paper describes the chemical and thermal properties of three fractions obtained by a two-stage dry fractionation of AMF. Fractions from a laboratory-scale process also are compared with those produced on a commercial scale. The relationship between the fatty acid and TAG composition and thermal properties of the fractions has been investigated.

---

*To whom correspondence should be addressed at Department of Food Science, 116 Borland Laboratory, The Pennsylvania State University, University Park, PA 16802.

[1] Current address: Lipid Technology Department, Central Food Technological Research Institute, Mysore, 570 013, India.

EXPERIMENTAL PROCEDURES

Fractionation of AMF. Commercial AMF (Tedford/Tellico, Concord, TN) was heated to 60°C, anhydrous sodium sulfate (Fisher Scientific, Pittsburgh, PA) was added, and the fat was filtered through filter paper (Whatman No. 1; Whatman, Clifton, NJ) to produce a clear fat that was free of moisture. This AMF was used for the laboratory dry fractionation. Three commercial milk-fat fractions with melting points of 41, 32, and 22°C (MP 41, MP 32, MP 22, respectively), were isolated on the Tirtiaux dry fractionation system with a florentine filter (Tirtiaux, s.a. Fractionnement, Fleurus, Belgium) and were supplied by E. Deffense (Deffense, E., 1993, personal communication. s.a. Fractionnement Tirtiaux) for comparison with AMF fractions prepared in the laboratory.

The AMF was fractionated in the laboratory by a two-stage dry fractionation process (Fig. 1). The fat was heated at 60°C to destroy crystal memory, cooled to 29°C at 1°C/min, and held at this temperature for 6 h with constant stirring (60 to 80 rpm). The solid (first stearin), high-melting fraction (HMF), was then removed from the liquid (first olein fraction) by filtration through Whatman No. 1 filter paper in a Buchner funnel under mild suction. The first olein fraction was reheated to 50°C, cooled to 18°C at 1°C/min, and held for 6 h to produce a second stearin fraction, designated as the middle-melting fraction (MMF) of AMF, and a second olein fraction, designated as the low-melting fraction (LMF) of AMF, which were separated by filtration under mild suction.

Thermal analysis. Differential scanning calorimetry (DSC) (model 7; Perkin-Elmer Corp., Norwalk, CT) was used to study the thermal behavior of the AMF and its fractions and to determine the solid fat content (SFC) at various temperatures. DSC also yielded information of the polymorphic forms, based on the number of endothermic peaks generated under different tempering conditions (15). The DSC was calibrated with an Indium standard (m.p. 156.6°C, ΔH_f 28.45 J/g). About 5-mg portions of the fat samples were accurately weighed into aluminum pans and heated at 60°C for 30 min to destroy crystal memory. Samples were tempered before thermal analysis by holding them at 0°C for 90 min and then raising the temperature to 26°C for 40 h, followed by lowering the temperature to 0°C for 60 min. Thermograms were generated from −20° to 45°C at 10°C/min and at 20°C/min to determine the effect of heating rate on the endothermic peak melting temperatures and on ΔH_f. SFC at various temperatures was calculated from the melting thermograms with the Perkin-Elmer (Norwalk, CT) partial area program. To obtain crystallization curves by DSC, samples were heated to 60°C for 10 min, cooled rapidly at 20°C/min to −20°C, heated from −20 to 60°C for 10 min, and cooled slowly at 5°C/min to −20°C to produce thermograms.

TAG composition. Analyses of the TAG were carried out on a 15 m × 0.25 mm capillary fused-silica column, coated with 0.1-mm bonded methyl 65% phenyl silicone (Quadrex Corporation, New Haven, CT). The column was installed in a model 5890 Hewlett-Packard GC (Hewlett-Packard Co., Avondale, PA), equipped with a flame ionization detector (FID) and a split injector. Flow rate of carrier gas (He) was 1.5 mL/min for the column and 65 mL/min for the split vent. Temperatures of the detector and injector were 380 and 320°C, respectively. Column temperature was programmed from 260 to 360°C at 3°C/min and held for 10 min isothermally at 360°C. Identification of individual TAG according to carbon number and double bonds (CN-NUFA) (17) was made by comparison of retention times with those of standard mixtures and also by cochromatography of selected standards with the sample.

Fatty acid composition. Samples were saponified with 1N alcoholic KOH at 75°C for 3 h. The contents were cooled, water was added, and the unsaponifiable matter was removed by extraction with diethyl ether. The aqueous portion was acidified with 5N HCl, and the fatty acids were extracted with diethyl ether. The organic layer was washed with water and dried over anhydrous sodium sulfate. Solvent was removed under nitrogen, and the residue was dissolved in $CHCl_3$. A Perkin-Elmer model 8410 GC, equipped with an FID, was used. Fatty acids were analyzed under the following conditions: 10% SP-216-PS coated on 100/120 Supelcoport (Supelco, Bellefonte, PA) glass column (0.61 m × 2 mm); column temperature 130 to 205°C at 15°C/min; carrier gas (N_2) at 20 mL/min; injector temperature 205°C; and detector temperature 250°C. The fatty acids were identified by comparing retention times with those of standards.

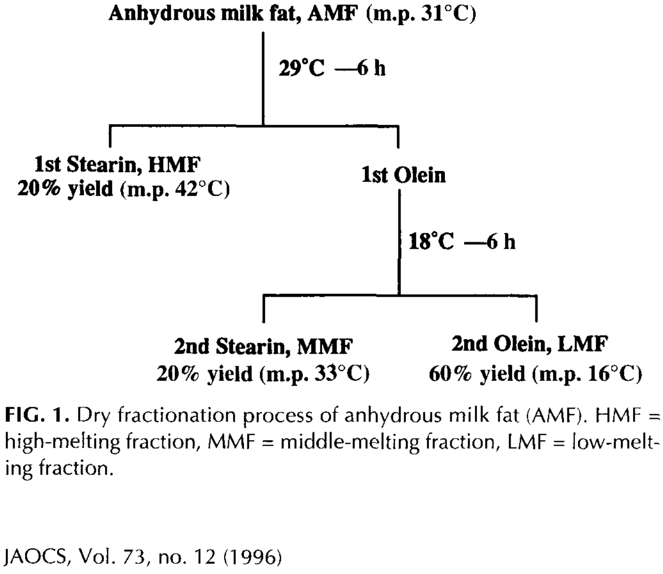

FIG. 1. Dry fractionation process of anhydrous milk fat (AMF). HMF = high-melting fraction, MMF = middle-melting fraction, LMF = low-melting fraction.

RESULTS AND DISCUSSION

Solidification profiles for the laboratory and commercial AMF fractions are illustrated in Figure 2. SFC obtained by DSC is a relative measure, and we assume 100% crystallinity at −20°C. SFC curves of the high-melting fractions (HMF and MP 41) and the lower-melting fractions (LMF and MP 22) were similar, independent of the method of fractionation. The HMF contained solid fat >40°C, indicating their potential to have a waxy mouthfeel. LMF and MP 22 were liquid at ambient temperature. The MMF and the commercial MP 32 demonstrated different SFC

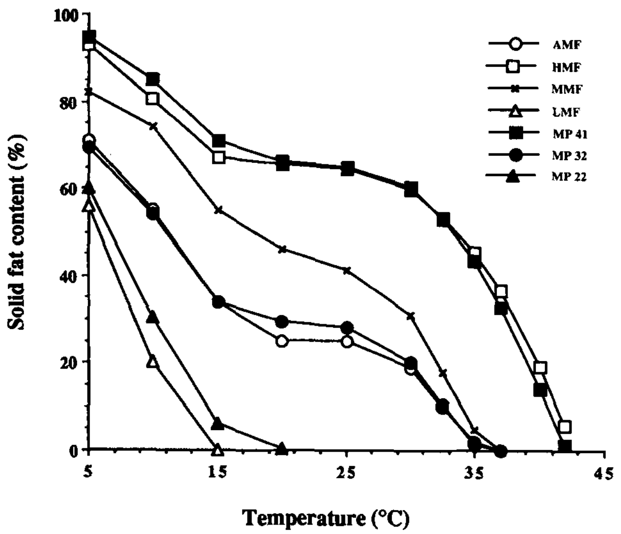

FIG. 2. Solid fat content of anhydrous milk fat (AMF), its fractions (HMF, MMF, LMF), and commercial milk-fat fractions (MP 41, MP 32, MP 22), obtained after tempering at 26°C.

profiles. The melting profile of MMF lies between those of HMF and AMF, possibly because of the differences in the AMF source. At 25°C, HMF contained 66% solids, MMF 43% solids, and LMF 0% solids. Unlike HMF, MMF possessed a considerable proportion of liquid fat <25°C and no solids >37°C.

Figure 3 illustrates the DSC endotherms of AMF and its fractions tempered at 26°C. The melting behaviors of the laboratory and commercial fractions were similar. AMF differed markedly from those of HMF and LMF and only slightly from that of MMF. HMF and MP 41 had a major endotherm at 42°C and a broad melting range with considerably higher SFC at higher (>30°C) temperatures, similar to that of a plastic fat, which is in accord with reports that milk fat crystallizes rapidly to the stable β′ form, and the high-melting acylglycerols crystallize independently of the bulk of the milk fat (18). The rate of heating after tempering at 26°C did not significantly influence endothermic peak temperatures or ΔH_f. HMF contained some entrapped low-melting fat, as evidenced by the endotherms between 7 and 14°C. LMF, laboratory and commercial, had a major endotherm at 12°C and were void of endotherms at temperatures greater than 22°C. Fractions isolated by the laboratory process were similar in thermal (Fig. 3) and TAG composition (Table 1) to those fractionated by the commercial process. Thus, discussion of the results centers on the analyses of the laboratory-prepared fractions.

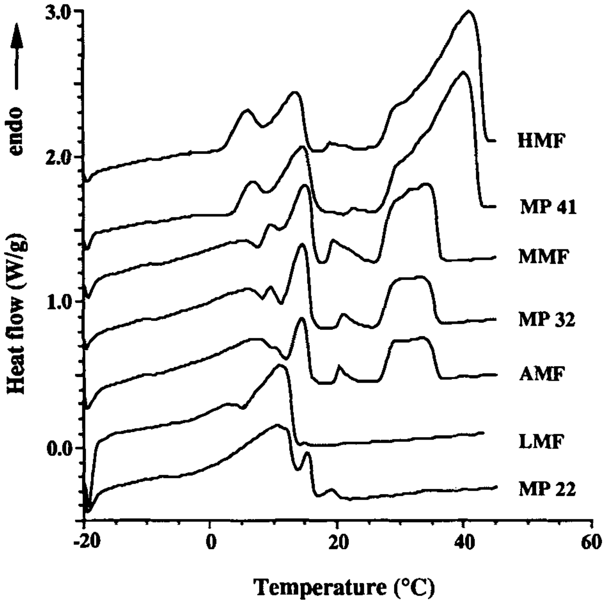

FIG. 3. Differential scanning calorimetric thermograms (10°C/min) of anhydrous milk fat (AMF), its laboratory fractions (HMF, MMF, LMF), and commercial fractions (MP 41, MP 32, MP 22), after tempering at 26°C.

ΔH_f of HMF is considerably higher (77.1 J/g) than that of the original AMF (47.2 J/g), and ΔH_f of LMF (36.5 J/g) was, as expected, lower than that of AMF (Table 2). ΔH_f and SFC of the MMF (59.5 J/g) were slightly higher than those of the original AMF, but the shape of the thermograms was similar. Thus, by two-stage fractionation, three fractions were produced that had entirely different melting behaviors.

Figure 4 illustrates the crystallization exotherms for AMF and its fractions, obtained by cooling from 60 to −20°C. The HMF crystallized at a much higher temperature (23°C) than the original AMF and other fractions. HMF showed two distinct exotherms: a sharp peak at higher temperature (23°C) and broad peaks at 5–10°C, similar to that of AMF and MMF. AMF, MMF, and LMF did not have an exotherm at the higher temperature. The exotherm at the higher temperature (23°C) is due to the high-melting TAG present in HMF. AMF and MMF showed two partly resolved exotherms during rapid cooling (20°C/min) that were resolved during slow cooling (5°C/min). The onset of crystallization of all samples shifted to higher temperatures during slow cooling, as illustrated by comparison of the HMF sample at the two cooling rates.

Blending of AMF, MMF, and LMF with HMF lowered the SFC of the latter (Fig. 5). LMF reduced the SFC of HMF as the concentration of LMF increased. The SFC of mixtures of HMF and LMF were lower than the corresponding blends of HMF with MMF or AMF. The SFC of binary mixtures of HMF and AMF or MMF increases in proportion to the content of HMF, illustrated in the isothermal solids diagram (Fig. 6). Addition or subtraction of small amounts of HMF has been shown by deMan (13) to result in substantial changes in the SFC of milk fat. Timms (19) observed that the addition of HMF increased the SFC, but the increase was not significantly different because the amount of HMF added was small.

The distinction between the physical characteristics of milk-fat fractions is due mainly to differences in distribution of TAG (Fig. 7). Trisaturated TAG of medium or high molecular mass (CN 36 to 54) were more concentrated in HMF, and unsaturated TAG in LMF. TAG with low (CN 24 to 34) and

TABLE 1
Triacylglycerol Composition of Laboratory and Commercial Milk-Fat Fractions[a]

	Laboratory			Commercial		
Triacylglycerol type[b]	HMF	MMF	LMF	MP 41	MP 32	MP 22
C24–34	7.6	10.2	12.2	6.0	11.7	19.7
C36–40	27.4	31.8	42.3	39.1	34.2	43.2
C42–54	65.0	57.9	45.5	54.5	53.6	36.8
C42–54 saturated	26.3	16.2	3.6	21.0	11.7	3.9
C42–54 unsaturated	32.8	35.8	37.0	25.3	32.7	29.2
C36–54 saturated	27.8	18.2	5.5	24.7	13.3	6.4
C36–54 unsaturated	51.2	58.2	71.7	53.5	62.8	62.9

[a] HMF = high-melting, MMF = middle-melting, and LMF = low-melting fraction; MP 41, MP 32, MP 22 = melting points of commercial fractions.
[b] Aggregate carbon number of triacylglycerols.

TABLE 2
Thermal Properties of Anhydrous Milk Fat and Its Fractions[1]

Milk fat	Enthalpy (J/g)	Peak melting temperature (°C)
Anhydrous milk fat	42.2 ± 2.0	33.1 ± 0.3
High-melting fraction	77.1 ± 0.09	41.6 ± 0.1
Middle-melting fraction	59.5 ± 1.3	34.1 ± 0.2
Low-melting fraction	36.5 ± 1.1	10.9 ± 0.1

[1] Mean and SD values of 4 replicate samples fractionated by the laboratory method.

medium (CN 36 to 40) carbon numbers were greater in AMF, MMF, and LMF than in HMF. The distribution of total saturated and unsaturated TAG with CN 36 to 54 were significantly different among the three fractions ($P < 0.05$). The proportion of saturated TAG decreased in order from HMF to MMF to AMF and to LMF. The fatty acid composition revealed that the long-chain saturated fatty acids (palmitic and stearic) were more concentrated in HMF, and the other fractions had higher content of unsaturated fatty acids (Fig. 8). Because of the presence of TAG that contained long-chain saturated fatty acids, HMF crystallized at higher temperatures and had high SFC.

HMF has the potential to be used in puff pastries because of its wide plastic melting point range. Because the higher-melting fractions of milk fat inhibit bloom formation in chocolate more effectively than original AMF (6,7), HMF could be used in chocolate for this purpose. LMF may find application in confectionery products to yield better eating qualities of soft centers and in frozen confectionery chocolate coatings. LMF has applications in carrying butter flavor into fluid cooking oils, and by blending LMF with HMF, a product with better spreadability at refrigeration temperatures than butter could be obtained. MMF, being similar to original milk fat, could be used in place of milk fat.

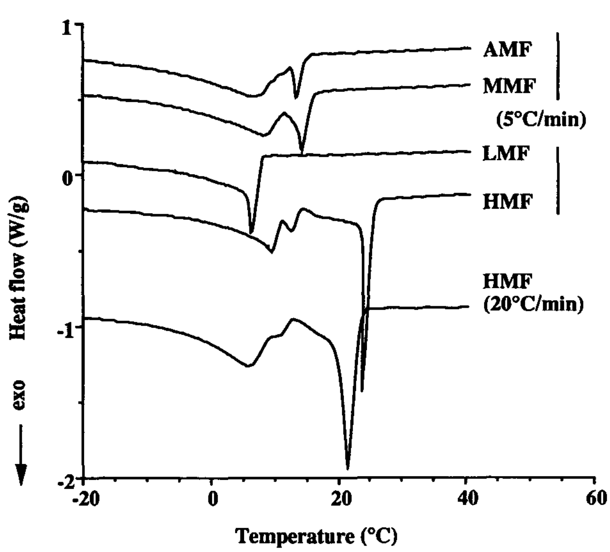

FIG. 4. Differential scanning calorimetric thermograms (10°C/min) of anhydrous milk fat (AMF) and its fractions (HMF, MMF, LMF), after slow (5°C/min) and rapid (20°C/min) cooling from 60 to −20°C.

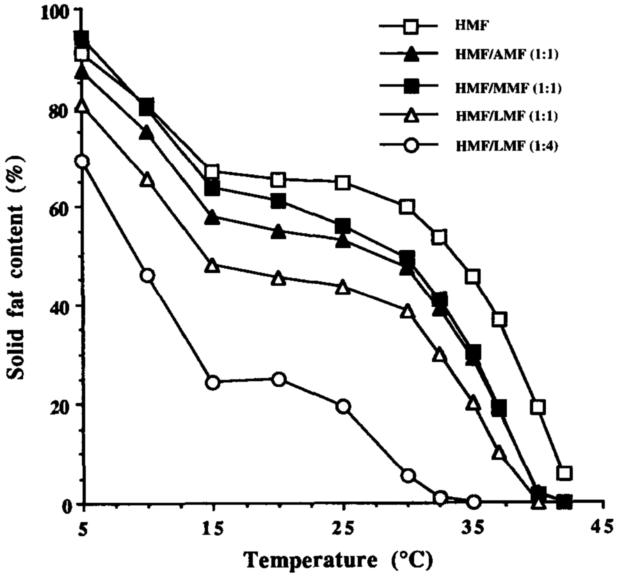

FIG. 5. Melting profiles of blends of high-melting fraction (HMF) with anhydrous milk fat (AMF), middle-melting fraction (MMF), and low-melting fraction (LMF).

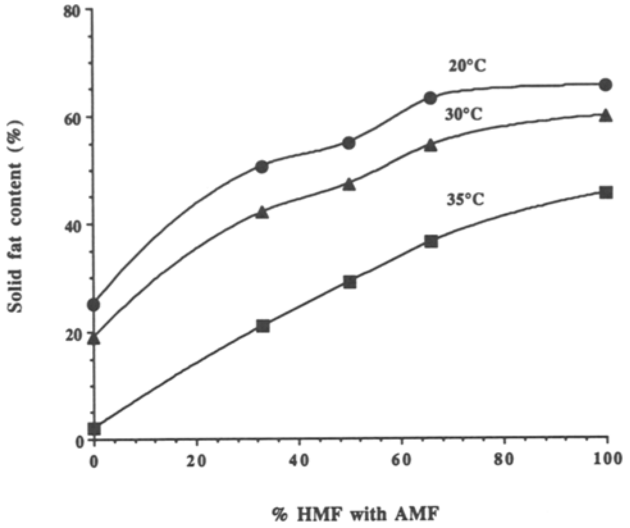

FIG. 6. Isothermal solid diagrams for binary mixtures of high-melting fraction (HMF) and anhydrous milk fat (AMF).

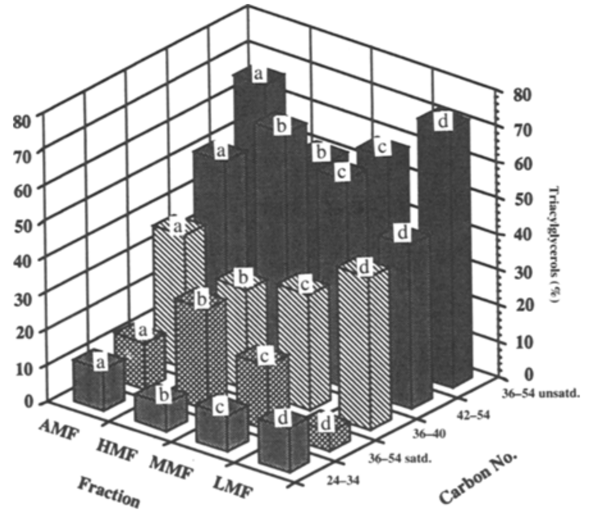

FIG. 7. Triacylglycerols composition of anhydrous milk fat (AMF) and its fractions (HMF, MMF, LMF) determined by capillary gas chromatography. Comparisons are between means of quadruplicate samples of each fraction. Bars with different letters are different (α = 0.05). satd = saturated triacylglycerols, unsatd. = unsaturated triacylglycerols.

ACKNOWLEDGMENTS

The authors gratefully acknowledge E. Deffense, Tirtiaux, S.A. Fractionnement, Fleurus, Belgium, for graciously supplying the commercial AMF fractions. This work was supported in part by the National Dairy Promotion and Research Board, Arlington, VA.

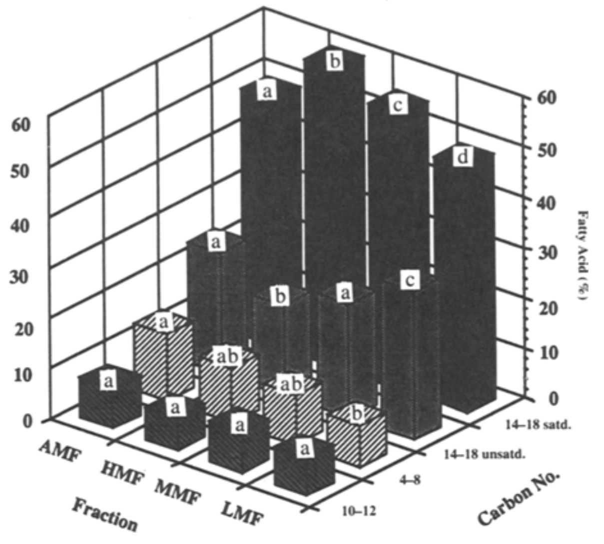

FIG. 8. Fatty acid composition of anhydrous milk fat (AMF) and its fractions (HMF, MMF, LMF). Comparisons are between means of triplicate samples of each fraction. Bars with different letters are different (α = 0.05). satd. = saturated fatty acids, unsatd. = unsaturated fatty acids.

REFERENCES

1. Barna, C.M., R.W. Hartel, and S. Martin, Incorporation of Milk Fat Fractions in Milk Chocolate, *The Manufacturing Confectioner* 72:107–116 (1992).
2. Deffense, E., Milk Fat Fractionation Today, A Review, *J. Am. Oil Chem. Soc.* *70*:1193–1201 (1993).
3. Kaylegian, K.E., and R.C. Lindsey, *Handbook of Milkfat Fractionation Technology and Applications*, AOAC Press, Champaign, 1995, pp. 1–18, 525–630.
4. Timms, R.E., and J.V. Parekh, The Possibilities for Using Hydrogenated, Fractionated or Interesterified Milk Fat in Chocolate, *Lebensm. Wiss. U. Technol.* *13*:177–181 (1980).
5. Versteeg, C., L.N. Thomas, Y.L. Yep, M. Papalois, and P.S. Dimick, New Fractionated Milk Fat Products, *Aust. J. Dairy Technol.* *49*:57–61 (1993).
6. Dimick, P.S., L.N. Thomas, and C. Versteeg, Potential Use of Fractionated Anhydrous Milk Fat as a Bloom Inhibitor in Dark Chocolate, *INFORM 4*:504 (1993).
7. Full, N.A., S.Y. Reddy, P.S. Dimick, and G.R. Ziegler, Physical and Sensory Properties of Milk Chocolate Formulated with Anhydrous Milk Fat Fractions, *J. Food Sci.* *61*:1068–1072, 1084 (1996).
8. Amer, M.A., D.B. Kupranycz, and B.E. Baker, Physical and Chemical Characteristics of Butterfat Fractions Obtained by Crystallization from Molten Fat, *J. Am. Oil Chem. Soc.* *62*:1551–1557 (1985).
9. Bhaskar, A.R., S.S.H. Rizvi, and J.W. Sherbon, Anhydrous Milk Fat Fractionation with Continuous Counter-Current Supercritical Carbon Dioxide, *J. Food Sci.* *58*:748–753 (1993).
10. Black, R.G., Partial Crystallization of Milk Fat and Separation of Fractions by Vacuum Filtration, *Aust. J. Dairy Technol.* *30*:153 (1975).
11. Dimick, P.S., and S. Patton, Structure and Synthesis of Milk Fat. VII Distribution of Fatty Acids in Milk Fat Triglycerides with Special Reference to Butyrate, *J. Dairy Sci.* *48*:444–449 (1965).
12. Timms, R.E., The Possibilities for Using Modified Milk Fats in the Production of Confectionary Fats, Shortenings and Spreads, in *Fats for the Future*, edited by R.C. Cambie, Ellis Horwood Ltd., Chichester, 1989, pp. 251–261.
13. deMan, J.M., Physical Properties of Milk Fat. II. Some Factors Influencing Crystallization, *J. Dairy Res.* *28*:117–122 (1961).

14. Deffense, E., Multi-Step Butteroil Fractionation and Spreadable Butter, *Fat Sci. Technol. 13*:502–507 (1987).
15. Huyghebaert, A., and H. Hendrickx, Polymorphism of Cocoa Butter Shown by Differential Scanning Calorimetry, *Lebensm. Wiss. U. Technol.*. *4*:59–63 (1971).
16. Belousov, A.P., and V.M. Vergelesov, On Certain Peculiarities of Crystallization of Milk Fat in Cooling, *XVI International Dairy Congress*, International Dairy Federation, Brussels, Belgium, 1962, pp. 49–60.
17. Kalo, P., and A. Kemppinen, Mass Spectrometric Identification of Triacylglycerols of Enzymatically Modified Butterfat Separated on a Polarized Phenylmethylsilicone Column, *J. Am. Oil Chem. Soc. 70*:1209–1217 (1993).
18. Tverdokhkleb, G.V., and V.M. Vergelesov, Factors Controlling Crystallization of Milk Fat, *XVIII International Dairy Congress*, International Dairy Federation, Brussels, Belgium, 1970, p. 211.
19. Timms, R.E., The Phase Behaviour and Polymorphism of Milk Fat, Milk Fat Fractions and Fully Hardened Milk Fat, *Aust. J. Dairy Technol. 35*:47–53 (1980).

[Received January 16, 1996; accepted July 11, 1996]