A Comparison of Physical and Chemical Properties of Milk Fat Fractions Obtained by Two Processing Technologies

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ABSTRACT: Milk fat fractions from supercritical carbon dioxide (SC-CO_2) extraction were compared with commercial melt crystallization (MC) fractions for their physical and chemical properties. The fractions were analyzed for fatty acids, triacylglycerols, cholesterol, total carotenoid content, and volatile compounds. The fractions were also evaluated for solid fat content (SFC) by pulsed nuclear magnetic resonance and thermal profiles by differential scanning calorimeter (DSC). The distribution of fatty acids and triacylglycerols in the fractions depended on the fractionation technique used. SC-CO_2 separated fractions based on molecular weight rather than on melting point, which is the driving force for the MC process. The differences among the fractions were quantified from their SFC and DSC curves. Triacylglycerol profiles by high-performance liquid chromatography showed that the SC-CO_2 fractions were distinctly different from each other and from MC fractions. The SC-CO_2 solid fraction (super stearin) was the most unique. It had a high concentration of long-chain, unsaturated fatty acid-containing triacylglycerols in a narrow range of high molecular weight, indicating a homogeneity of this fraction that has not been attainable by other techniques. It was also enriched in β-carotene and was devoid of volatile compounds. As compared to liquid MC fractions, the liquid SC-CO_2 fraction had a high concentration of low-melting triacylglycerols and was enriched in volatile compounds. With SC-CO_2, it is thus possible to simultaneously fractionate and produce a flavor-rich concentrate at no extra processing cost.

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KEY WORDS: Cholesterol, DSC, fatty acids, flavor, fractionation, melt crystallization, milk fat, solid fat content, supercritical CO_2, triacylglycerol.

New food products are being developed at a rapid rate and place high demands for more specialized fats and oils, customized to fit specific applications. Optimal fats, however, cannot always be obtained from nature. Milk fat can be singled out from most other fats in that it has a broad molecular weight range of triacylglycerols (TAG), low (17 wt%), medium (50 wt%) and high (33 wt%), coupled with a pleasing aroma and taste (1). But, when these TAG are viewed as a single entity, they can and do limit the use of milk fat. Price and functionality are important factors that affect usage of milk fat: milk fat has the disadvantage of both high price and limited functionality compared to tailored vegetable fats and oils. Its flavor and its reputation as a natural product are the biggest advantages of milk fat. Moreover, milk fat is required for flavor in certain applications and high-quality foods, e.g., premium cookies and bakery products.

Given milk fat's limited functional and nutritional value, its enhanced utilization hinges on providing unique functionality through fractionation. The most common method of milk fat fractionation is by crystallization at different temperatures (melt crystallization, or MC), with or without solvents (2–5). The fractions obtained by dry fractionation, however, show differences in melt characteristics but relatively small variations in chemical composition (1). The use of solvents or surfactants improves separation by reducing entrainment, but these techniques are not environmentally friendly owing to problems related to solvent removal and disposal. The fractions obtained have to be deodorized, which results in the loss of natural flavor compounds. To maximize milk fat utilization, the economics of the process dictate that the fractions must differ markedly from one another in both chemical composition and physical characteristics. This approach calls for advanced separation technologies for new product and process development, aimed at the needs of existing and new markets.

Supercritical carbon dioxide (SC-CO_2) fractionation holds promise as a means to turn milk fat into a value-added ingredient (6–8). So far, no comparative evaluation of physical and chemical properties of the milk fat fractions from these two techniques (MC and SC-CO_2) has been undertaken. This paper compares data from these two techniques.

METHODS

Fractionation by MC. Four different kinds of commercial MC fractions (AMF45, AMF30, AMF20, and AMF10) were obtained directly from S.A.N. Corman, Goé, Belgium. The fractions varied in their melting points from 45 to 10°C. The details of the process conditions and yields of the fractions were not available. Further, each fraction could have been prepared from an individual batch.

Fractionation by SC-CO_2. The SC-CO_2 fractions were prepared in an in-house designed and built continuous pilot-

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TABLE 1
Operating Conditions for Milk Fat Fractionation with Supercritical CO_2 (SC-CO_2)

Solubility (wt%): 1.56–1.58
Solvent-to-feed ratio (g/g): 57–60

Parameters	Feed milk fat	Column super stearin	Separator 1 stearin	Separator 2 olein	Separator 3 super olein	Cold trap
Pressure (MPa)	24.1	24.1	17.2	10.3	6.9	0.03
Temperature (°C)	40	40	50	40	60	4
Yield (wt%)	100	12	33	35	19	1

scale SC-CO_2 system. The extraction was done at 24.1 MPa/40°C, and fractionation pressures and temperatures ranged from 17.2 to 6.9 MPa and 40 to 60°C, respectively. Table 1 shows the details of the fractionation conditions, along with the yields for the fractions. More details about the system have been published elsewhere (9).

Analyses. TAG spectra of the milk fat fractions were analyzed by both gas–liquid chromatography (GLC) and reversed-phase high-performance liquid chromatography (HPLC). The GLC analysis was done on a GC 8000 series chromatograph (Fisons Instruments, Rodano, Italy), equipped with flame-ionization detector (FID) (held at 380°C) and on-column injector. A fused-silica capillary column Permabond OV-1-DF-0.10, 10 m × 0.32 mm i.d., film thickness 0.12 μm (Macherey-Nagel, Düren, Germany), was used. Data acquisition was carried out with a Spectra Physics SP4100 integrator (Allschwil, Switzerland). About 15 mg fat was dissolved in 100 mL hexane (cat. no 9262, J.T. Baker B.V., Deventer, Netherlands). The following program allowed separation of the TAG: 80°C iso 2 min, 5°C/min to 200°C, 1 min iso, 5°C/min to 310°C, and 17 min iso. Carrier gas: hydrogen at 60 kPa.

The reversed-phase HPLC analysis was done with a Waters system (625 LC system, 600 E system controller, 715 ultra wisp sample processor; Waters, Le Mont-sur-Lausanne, Switzerland), combined with an evaporative light scattering detector model ACS 750/14 from Ercatech AG, Bern, Switzerland. Data acquisition and integration were performed with the Maxima 825 software from Dynamic Solutions (Ventura, CA). About 200 mg of sample was dissolved in 10 mL of a 1:1 (vol/vol) blend of diethyl ether/toluene; injection volume was 10 μL. Separation was performed at ambient temperature with two columns of LiChrospher 100RP-18, 4 × 250 mm, 5 mm (E. Merck, Darmstadt, Germany) in series and a binary gradient of (i) acetonitrile/ethanol/hexane (2:2:1, vol/vol/vol) and (ii) acetonitrile. The linear gradient (1 mL/min) was 50% A/50% B to 100% B in 120 min. All solvents were of HPLC grade and were supplied by E. Merck and Fluka AG (Buchs, Switzerland).

Fatty acids were converted to fatty acid methyl esters (FAME) and analyzed by GLC on an HR5160 Mega Series gas chromatograph (Carlo Erba Instruments, Rodano, Italy), equipped with FID (held at 320°C) and on-column injector. Separation was achieved on a fused-silica capillary column DB-Wax (J&W Scientific, Folsom, CA), 30 m × 0.32 mm i.d., film thickness 0.25 μm. FAME were prepared as follows: add 1 mL methanolic HCl 3 N (Supelco, Buchs, Switzerland), 1 mL methanol p.a. (E. Merck) and 0.5 mL hexane (cat. no 9262, J.T. Baker B.V.) to about 10 mg fat, heat for 1 h at 100°C in a tightly closed glass vial, cool to ambient temperature, add 2 mL distilled water and 2 mL hexane per mg fat. Inject 1 μL of the supernatant (hexane phase) into the gas chromatograph. The following program allowed separation of the FAME: 40°C iso 2 min, 15°C/min to 145°C, 1 min iso, 3°C/min to 195°C, 5°C/min to 220°C, and 20–30 min iso. Carrier gas: hydrogen at 60 kPa.

Cholesterol was analyzed by following the method of Dieffenbacher *et al.* (10) with epicoprostanol (Sigma, Buchs, Switzerland) as an internal standard. GLC analyses were performed on a HRGC 4160 (Carlo Erba), equipped with on-column injector.

Total carotenoid content, calculated as β-carotene, was analyzed spectrophotometrically by the British Standards Institute method (11) (BS 684, section 2.20, 1977) for determination of carotene in vegetable oils.

The milk fat fractions from both techniques were analyzed for flavor volatiles, which were isolated by simultaneous distillation/extraction (SDE) according to Nickerson and Likens (12), with diethyl ether as solvent. About 10 g of each sample (except cold trap sample, which was 5 g) was mixed with 100 mL distilled water and boiled for 2 h. The solvent extracted was dried over anhydrous sodium sulfate and concentrated to 0.2 mL.

The volatiles were characterized in a Finnigan MAT (San Jose, CA) 8430 mass spectrometer coupled to a GLC (Hewlett-Packard 5890; Avondale, PA). A DB-1701 capillary column (J&W Scientic, Folsom, CA) was employed (30 m × 0.32 mm i.d., 0.25 μm film thickness) to separate the volatiles. The samples were introduced by cold on-column technique. The oven temperature was held at 35°C for 2 min, increased to 50°C at 40°C/min and held for 1 min, increased to 180°C at 6°C/min, and finally to 240°C at 10°C/min, and then held for 20 min.

The solid fat contents (SFC) of the fractions were measured by low-resolution pulsed nuclear magnetic resonance (NMR) spectroscopy in a minispec pc20 (Bruker Physik AG, Karlsruhe-Forchheim, Germany). The measurements were performed by the direct method with relaxation delay and enhancement values set at 2. The following thermal pretreatment was used for all milk fat fractions: samples in special NMR tubes were completely melted by heating to 90°C, and were

then cooled to 65°C. After 30 min, the samples were transferred to a 0°C water bath and held for 16 h. The SFC was then measured at 5°C intervals until complete melting. Samples were held for 30 min at each temperature. To study the effect of pretreatment on SFC content, data were also collected for samples after holding them at 0°C for 1, 2, 3, 16, and 24 h. For the 2-h holding time, the SFC was measured only at 0°C.

The melting profiles of the fractions were determined by differential scanning calorimetry (DSC) in a DSC-7 (Perkin-Elmer, Rotkreuz, Switzerland). The following temperature program was used: about 10 mg sample was melted at 60°C and held for 5 min before cooling to −50°C at the rate of 5°C/min. The samples were again held at this temperature for 5 min before heating to 60°C at the rate of 5°C/min.

RESULTS AND DISCUSSION

Physical and chemical properties of milk fat fractions. Tables 2 and 3 show the fatty acid distribution of milk fat fractions obtained by SC-CO_2 and MC. No data were available on the composition of milk fat used to obtain the MC fractions. The short- ($C_{4:0}$–$C_{8:0}$) and medium-chain ($C_{10:0}$–$C_{12:0}$) fatty acid concentration increased, and the long-chain ($C_{14:0}$– $C_{20:0}$) fatty acid concentration decreased from solid to liquid fractions (super stearin to super olein for SC-CO_2 and AMF45 to AMF10 for MC) for both techniques. However, when the fatty acids were separated as unsaturated and saturated, significant differences were seen in the two techniques. The total saturated fatty acid concentration increased, and the unsaturated fatty acid concentration decreased from solid to liquid for the SC-CO_2 fractions. This was the opposite of the MC fractions, where the liquid fractions (AMF20, AMF10) had a higher concentration of unsaturated fatty acids and lower concentration of saturated fatty acids. This was also seen in the unsaturated/saturated fatty acid ratio, which followed an opposite trend for the two techniques. The ratio decreased from solid to liquid for the SC-CO_2 fractions and increased for the MC fractions.

The TAG concentration (Tables 4 and 5), as determined by GLC, was also different for the fractions obtained by the two techniques. Compared to MC fractions, the SC-CO_2 liquid fractions had a higher concentration of low-molecular weight TAG (C_{24}–C_{34}) and medium-molecular weight TAG (C_{36}–C_{40}) and a lower concentration of the high-molecular weight TAG (C_{42}–C_{54}). Similarly, the super stearin SC-CO_2 fraction had a high concentration of high-molecular weight TAG, and little low-molecular weight TAG and medium-molecular weight TAG, as compared to the MC AMF45 fraction.

Figures 1–5 show the HPLC traces for milk fat and a few fractions. Milk fat was separated into 87 peaks, ranging from 0.1 to 8.1wt%. However, the equivalent carbon number (ECN —total number of carbon atoms of the three fatty acid moieties minus two times the number of double bonds) and fatty acid composition of the peaks were not determined. The retention times of the corresponding peaks fluctuated within a 30-s interval because the HPLC columns were not temperature-controlled. The two fractionation techniques did not modify the peak pattern as such. Major peaks in AMF (Fig.

TABLE 2
Fatty Acid Composition Percentage (area %) for SC-CO_2 Milk Fat Fractions by GLC

FAME[a]	Milk fat	Super stearin	Stearin	Olein	Super olein
$C_{4:0}$	3.39	0.45	2.86	4.64	5.95
$C_{6:0}$	2.11	0.22	1.88	2.73	3.28
$C_{8:0}$	1.28	0.20	1.17	1.53	1.91
$C_{10:0}$	2.85	0.70	2.72	3.25	3.90
$C_{12:0}$	3.20	1.23	3.08	3.52	4.14
$C_{14:0}$	10.43	6.37	10.29	11.12	12.18
$C_{14:1}$	0.91	0.54	0.91	0.97	1.00
$C_{15:0}$	0.94	0.69	0.93	0.98	1.03
$C_{16:0}$	28.73	24.99	28.94	29.48	29.55
$C_{16:1}$	1.41	1.25	1.46	1.41	1.37
$C_{17:0}$	0.55	0.63	0.56	0.50	0.50
$C_{18:0}$	10.34	15.64	10.51	9.20	8.16
$C_{18:1}$ *cis*	20.91	29.80	21.53	18.74	16.47
$C_{18:1}$ *trans*	2.13	3.09	2.17	1.89	1.64
$C_{18:2}$	2.64	3.30	2.76	2.47	2.16
$C_{18:3}$ α	0.46	0.74	0.51	0.41	0.40
$C_{20:0}$	0.12	0.31	0.11	0.08	0.06
$C_{20:1}$ n-9	0.11	0.23	0.15	0.10	0.08
Others	7.49	9.63	7.46	6.99	6.21
C_4–C_8	6.78	0.86	5.91	8.89	11.13
C_{10}–C_{12}	6.05	1.93	5.81	6.77	8.04
C_{14}–C_{20}	79.68	87.58	80.82	77.35	74.61
Saturated	63.94	51.41	63.06	67.02	70.66
Unsaturated	28.58	38.96	29.48	25.99	23.13
Unsat/sat	0.45	0.76	0.47	0.39	0.33

[a]Abbreviations: GLC, gas–liquid chromatography; FAME, fatty acid methyl ester; unsat/sat, saturated total area % unsaturated fatty acids)/(total area % saturated fatty acids). For other abbreviations see Table 1.

TABLE 3
Fatty Acid Composition Percentage (area %) for Melt Crystallization (MC) Milk Fat Fractions by GLC

FAME	AMF45[a]	AMF30[a]	AMF20[a]	AMF10[a]
$C_{4:0}$	3.08	3.44	4.45	4.67
$C_{6:0}$	1.80	2.20	2.65	2.89
$C_{8:0}$	1.11	1.37	1.50	1.76
$C_{10:0}$	2.74	3.07	3.21	3.83
$C_{12:0}$	3.88	3.76	3.89	4.81
$C_{14:0}$	11.88	11.04	10.72	10.87
$C_{14:1}$	0.75	0.98	0.54	1.32
$C_{15:0}$	1.16	1.13	1.03	0.94
$C_{16:0}$	34.38	29.43	29.63	22.27
$C_{16:1}$	1.19	1.53	1.68	2.21
$C_{17:0}$	0.64	0.62	0.56	0.42
$C_{18:0}$	11.81	9.86	8.60	6.08
$C_{18:1}$ n-9 *cis*	15.05	19.46	20.17	25.27
$C_{18:1}$ n-9 *trans*	1.75	2.40	1.69	1.88
$C_{18:2}$ n-6	1.04	1.19	1.48	1.90
$C_{18:3}$ n-3 α	0.30	0.57	0.49	0.59
$C_{20:0}$	0.19	0.15	0.10	0.06
$C_{20:1}$ n-9	0.14	0.16	0.16	0.17
Others	7.11	7.66	7.44	8.08
C_4–C_8	5.99	7.00	8.61	9.31
C_{10}–C_{12}	6.62	6.83	7.10	8.64
C_{14}–C_{20}	80.28	78.52	76.86	73.98
Saturated	72.67	66.06	66.35	58.59
Unsaturated	20.22	26.29	26.21	33.34
Unsat/sat	0.28	0.40	0.40	0.57

[a]MC fractions supplied by S.A.N. Corman, Groé, Belgium. For abbreviations see Table 2.

1) were also major peaks in the fractions. The most apparent difference in peak distribution was between the two hard stearins (AMF45 and super stearin). Their peak patterns were completely different. AMF45 (Fig. 2) was composed of peaks that eluted over the whole time range, whereas super stearin (Fig. 3) only showed peaks with retention times above 53 min. The liquid fractions (Figs. 3 and 5) showed only minor differences in their HPLC profiles. Only 10 peaks accounted for ≥ 3 wt% each and had a total of 50.2 wt% in milk fat. Almost half of all peaks were present in amounts below 0.5 wt%, indicating that the majority of TAG were present in only trace amounts. One peak does not represent one single TAG but rather a group with the same ECN (13).

The 10 most predominant peaks of milk fat and the four

TABLE 4
Percentage (area %) Triacylglycerol (TAG) Composition for SC-CO_2 Milk Fat Fractions by GLC[a]

TAG	Milk fat	Super stearin	Stearin	Olein	Super olein
C_{24}	0.27	0.07	0.16	0.33	0.64
C_{26}	0.26	0.00	0.00	0.12	0.68
C_{28}	0.47	0.00	0.16	0.42	1.60
C_{30}	0.44	0.00	0.43	1.12	2.72
C_{32}	2.05	0.00	1.08	2.49	4.59
C_{34}	4.60	0.13	3.05	5.90	8.75
C_{36}	9.02	0.53	7.09	11.68	14.53
C_{38}	11.73	0.86	10.53	15.06	16.34
C_{40}	9.51	1.14	9.90	12.02	11.92
C_{42}	6.17	1.46	6.95	7.44	6.63
C_{44}	5.65	2.46	6.76	6.31	5.00
C_{46}	6.16	5.07	7.55	6.16	4.77
C_{48}	7.89	10.58	9.41	6.63	5.13
C_{50}	10.70	21.46	11.48	7.50	5.86
C_{52}	11.85	31.01	11.41	6.66	4.87
C_{54}	5.57	18.54	4.88	2.80	1.89
Others	7.66	6.70	9.15	7.36	4.09
Sum (C_{24}–C_{34})	8.08	0.20	4.89	10.38	18.97
Sum (C_{36}–C_{40})	30.26	2.53	27.52	38.75	42.79
Sum (C_{42}–C_{54})	53.99	90.58	58.44	43.51	34.15

[a]For abbreviations see Tables 1 and 2.

TABLE 5
TAG (by GLC) Distribution (wt%) for MC Milk Fat Fractions[a]

TAG	AMF45	AMF30	AMF20	AMF10
C_{24}	0.22	0.31	0.34	0.67
C_{26}	0.13	0.26	0.29	0.39
C_{28}	0.35	0.53	0.49	0.86
C_{30}	0.78	1.06	1.24	1.96
C_{32}	1.68	2.30	2.74	3.69
C_{34}	3.96	5.29	6.35	6.90
C_{36}	7.86	9.87	12.12	9.98
C_{38}	9.24	11.80	13.95	13.42
C_{40}	7.68	9.22	10.38	10.91
C_{42}	6.63	6.39	6.71	6.27
C_{44}	7.31	5.83	5.31	5.44
C_{46}	8.62	6.18	5.19	5.45
C_{48}	10.14	7.51	6.33	6.03
C_{50}	11.89	9.80	8.48	6.82
C_{52}	9.30	9.98	8.53	8.34
C_{54}	2.86	4.64	3.87	4.43
Others	11.35	9.03	7.70	8.44
Sum (C_{24}–C_{34})	7.11	9.75	11.44	14.47
Sum (C_{36}–C_{40})	24.78	30.90	36.44	34.31
Sum (C_{42}–C_{54})	56.75	50.32	44.42	42.79

[a]For abbreviations see Tables 3 and 4. For supplier see Table 3.

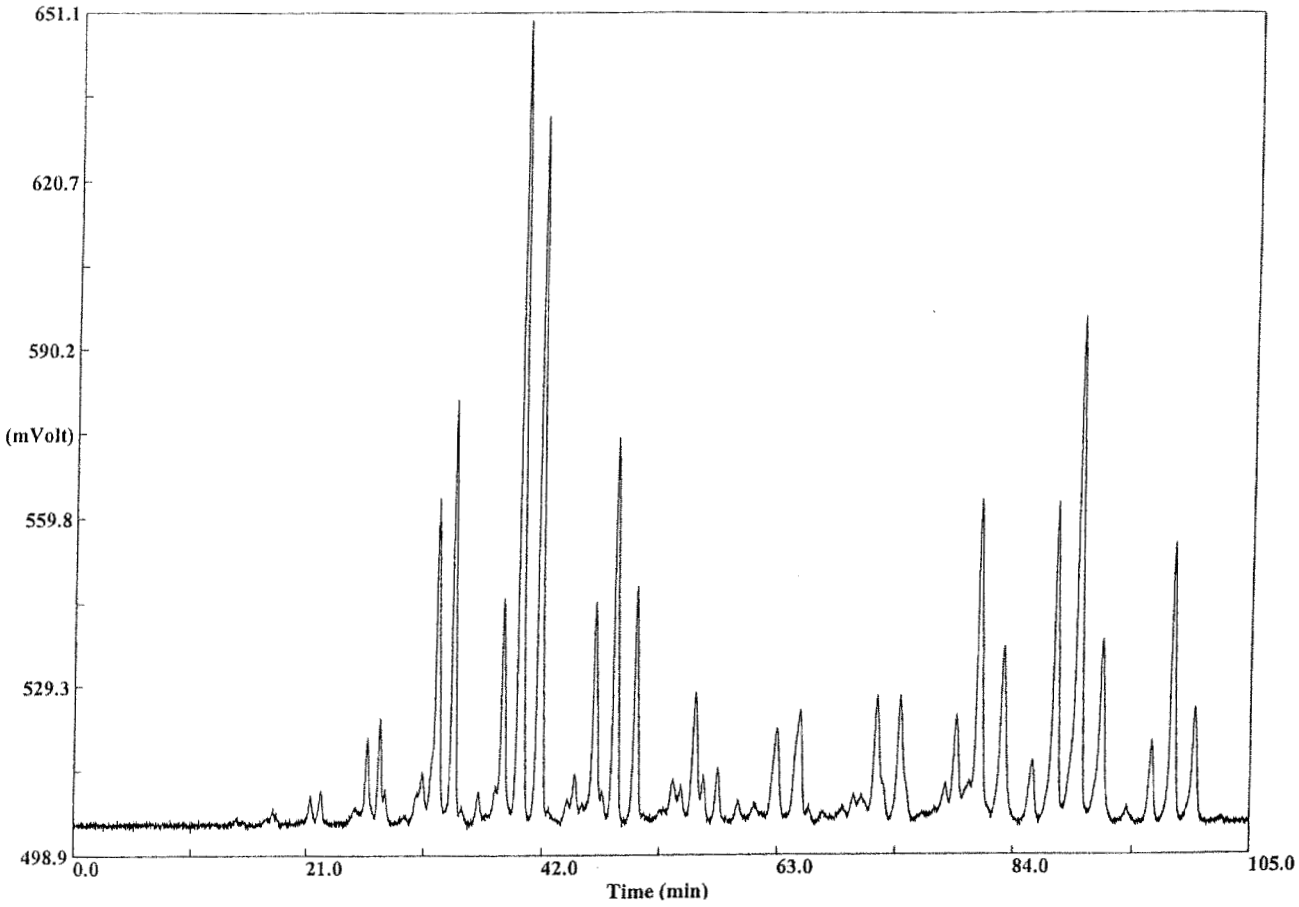

FIG. 1. High-performance liquid chromatography (HPLC) profile for milk fat (baseline not adjusted).

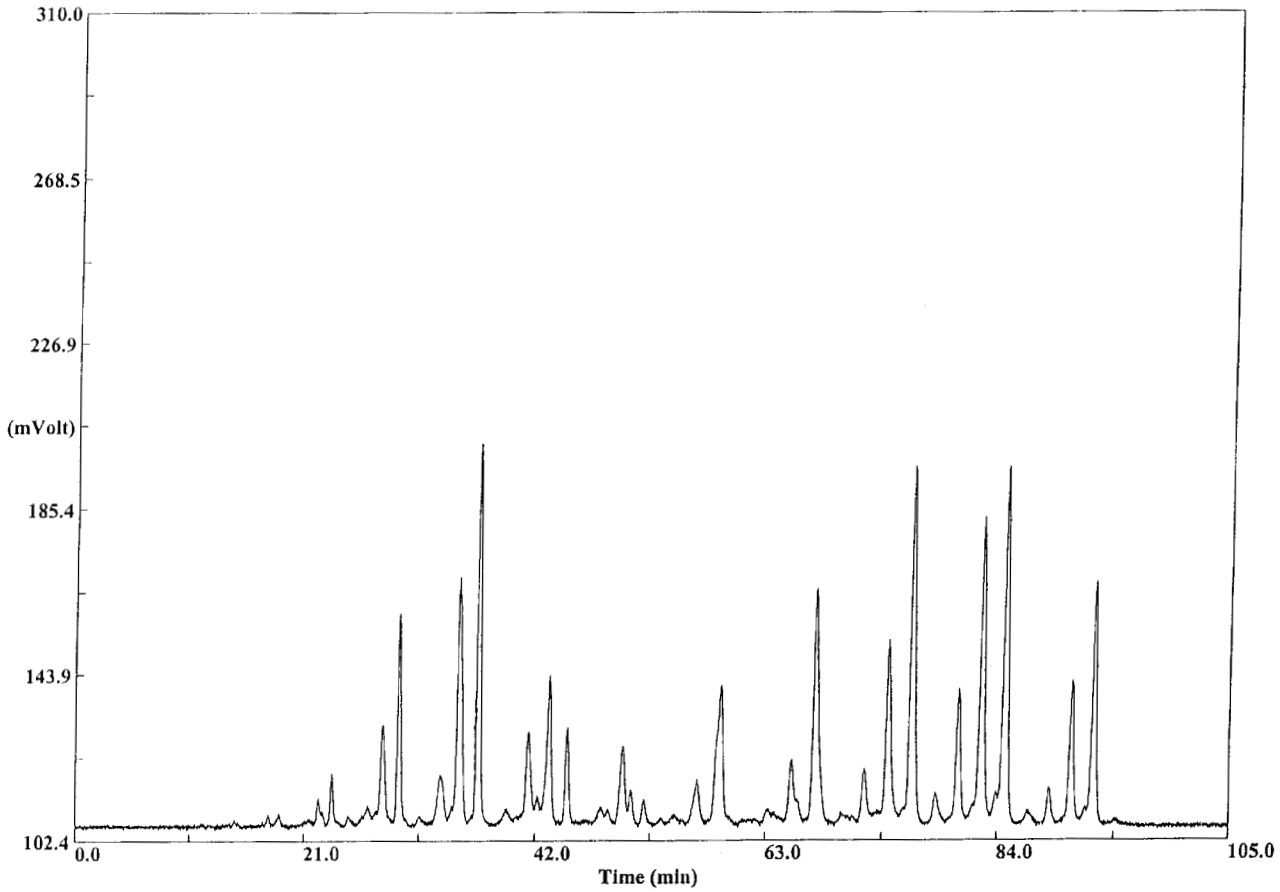

FIG. 2. HPLC profile for melt crystallization AMF45 (supplied by S.A.N. Corman, Groé, Belgium). For abbreviation see Figure 1.

$SC\text{-}CO_2$ fractions accounted for 50–60 wt% in total, except for super stearin in which they amounted to 80 wt%. The MC fractions were even more similar; the 10 most abundant peaks represented 55–57 wt% of all peaks. Peaks were detected between 17.5 and 96.0 min, except for super stearin, which showed no peaks before 53.0 min. The distinct separation capacity of $SC\text{-}CO_2$ was well illustrated by this peak distribution. Forty-three peaks, which accounted for 47.9 wt% of total TAG, were detected before 53 min in AMF. Peaks of high concentration occurred in two retention time intervals: between 15 and 45 min and after 73 min. In between, the biggest peaks did not exceed 1.7 wt% (only six out of 32 peaks above 0.8 wt%). This intermediate time interval represented more than one-third of all peaks (32 out of 87), but in terms of concentration only one-fifth (20.1 wt%). Enrichment factors (concentration of peak α in fraction *x* compared with the corresponding peak α in AMF) were calculated for the 10 most abundant peaks. Highest peak enrichment was achieved in super stearin (five peaks were three- to fourfold enriched), whereas in the other $SC\text{-}CO_2$ fractions, enrichment was not above two. In the four olein fractions, half of the peaks eluted before 45 min, and these summed up to more than half of the total peak concentration for those particular fractions. This was more pronounced for the $SC\text{-}CO_2$ than the MC samples (olein: 65.6 wt% $\alpha = 28$ and super olein: 72.9 wt% $\alpha = 47$ vs. AMF20: 56.9 wt% $\alpha = 39$ and AMF10: 60.4 wt% $\alpha = 41$).

Fractionation by $SC\text{-}CO_2$ is based on molecular weight and dielectric properties of the solute rather than on melting point, which forms the basis of the MC process. The TAG that contain unsaturated fatty acids have lower melting points than TAG that contain saturated fatty acids. Hence, the unsaturated fatty acid concentration increased in the MC liquid fractions. However, TAG with unsaturated fatty acids may not have low molecular weights. Therefore, the $SC\text{-}CO_2$ liquid fractions had less unsaturated fatty acids or TAG with unsaturated fatty acids.

Further, the fractions obtained with $SC\text{-}CO_2$ were continuously extracted (single-pass) whereas MC is a batch process. McCarthy *et al.* (14) have studied milk fat fractionation by molecular distillation in a batch process. The fatty acid and TAG distribution trend is similar to that after $SC\text{-}CO_2$. Their residue, obtained after two passes, had a composition similar to $SC\text{-}CO_2$ super stearin. The authors also redistilled the 10% most volatile fraction (D-1) to obtain four more fractions, which may not be comparable with results from single-pass distillations. In another study on distillation, Arul *et al.* (15) fractionated milk fat into four fractions by short-path distillation (SPD). Their liquid fractions had higher concentrations of short- and medium-chain fatty acids and short- and

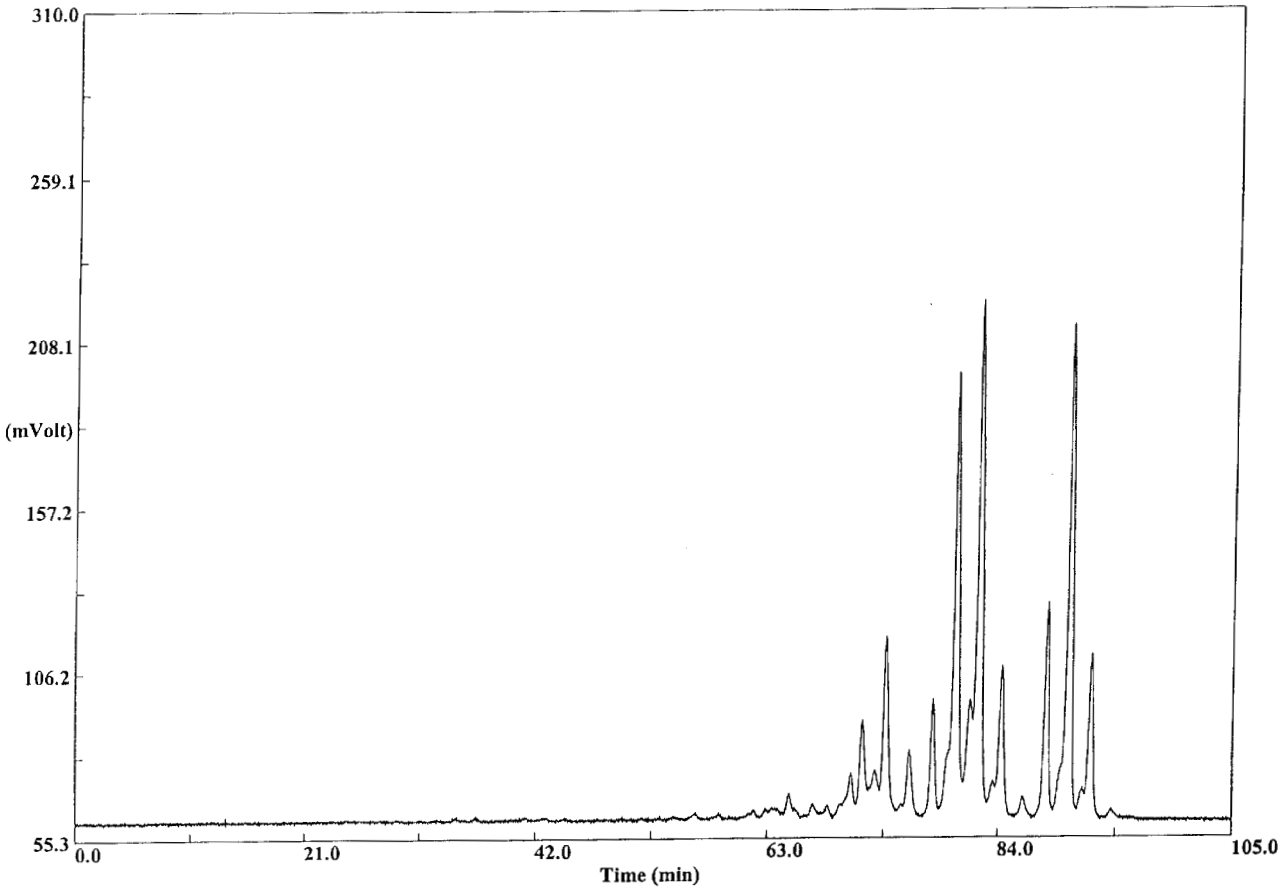

FIG. 3. HPLC profile for supercritical CO_2 (SC-CO_2) super stearin. See Figure 1 for abbreviation.

medium-chain TAG, compared to SC-CO_2 fractions. The combined liquid SPD fraction yield was 11.6%. The solid SPD fraction was different from SC-CO_2 super stearin.

Table 6 shows the cholesterol and β-carotene distribution among the milk fat fractions. The cholesterol concentration in the fractions followed a similar trend for the two techniques; as expected, it increased from solid to liquid fractions. The cholesterol concentration was reduced by more than 50% for the super stearin SC-CO_2 fraction. Further, the increase in cholesterol concentration was higher for the liquid MC fractions, compared to the liquid SC-CO_2 fractions. Cholesterol tends to concentrate in the more soluble fractions (liquid), which may be the result of a higher affinity of cholesterol for the short- and medium-chain fatty acids (4).

The β-carotene concentrations in the MC fractions were almost the same, whereas the SC-CO_2 fractions showed a distribution. The super stearin SC-CO_2 fraction had four times the β-carotene concentration of normal milk fat, whereas the liquid fractions had low concentrations.

Similar distribution trends for fatty acids, TAG, and cholesterol have been observed by other researchers (4,6,9,16).

Figures 6–11 show profiles for volatile compounds detected in milk fat and the solid and liquid fractions from the two techniques. Because the analysis was qualitative, the concentrations of the different volatiles could not be calculated. The fractions from both techniques show a distribution in lactone concentration. Lactones are important constituents of the unique butter flavor (17). The milk fat had only small amounts of different lactones, indicated by the small peaks on the chromatograms (Fig. 6). For the MC fractions, all fractions showed a presence of lactones, with the solid fraction (AMF45, Fig. 7) having the lowest and the liquid fraction (AMF10, Fig. 8) the highest concentrations, as seen by the peak heights. The concentration distribution of the lactones was different for the SC-CO_2 fractions. The super stearin fraction (Fig. 9) had no lactones, while the stearin and olein fractions had only low concentrations. However, the concentration increased in the super olein fraction (Fig. 10) and even more significantly in the flavor concentrate fraction (cold trap, Fig. 11). The flavor profile of the flavor concentrate had more than five times the concentration of lactones, compared to normal milk fat.

Figures 12A and B show the DSC curves for the milk fat fractions. All curves have three distinct peaks, which corresponded to low-melting (LMT), medium-melting (MMT), and high-melting (HMT) TAG. The SC-CO_2 super stearin fraction had a more distinct shoulder plateau, compared to AMF45, which agrees with the compositional differences for these fractions. This shoulder plateau decreased for the liquid fractions in both techniques. Both AMF20 and AMF10 frac-

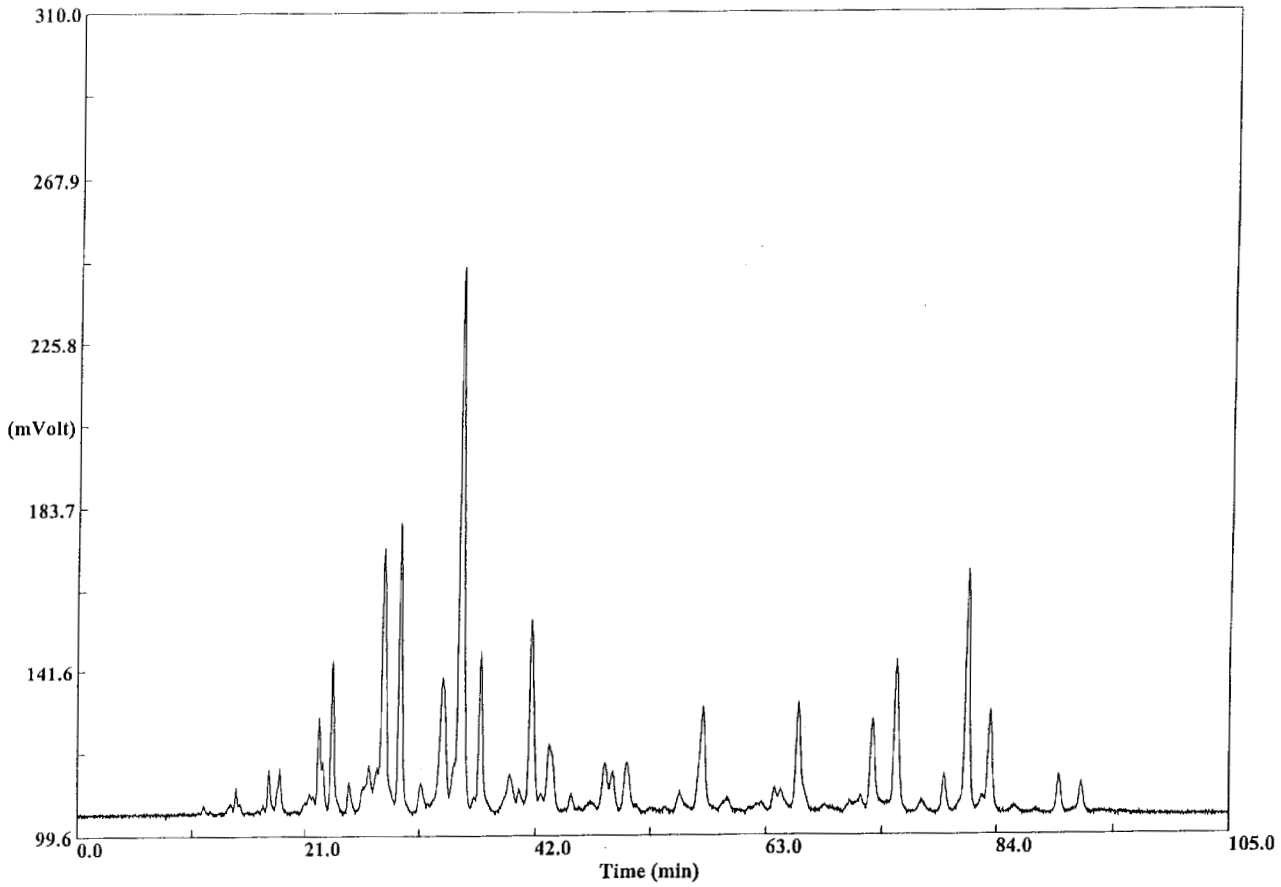

FIG. 4. HPLC profile for melt crystallization AMF10. See Figure 1 for abbreviation.

tions, however, showed no peak for HMT, even though their compositions showed the presence of HMT. This peak was small for the liquid SC-CO_2 fractions.

Figures 13A and B show the SFC profiles for milk fat fractions tempered at 0°C for 16 h. The profiles were again similar for the two techniques, but all SC-CO_2 fractions were different from milk fat. The AMF30 fraction had a profile similar to milk fat, and AMF10 had the lowest melting point of all fractions (SC-CO_2 and MC).

Table 7 shows the SFC data measured by pulsed NMR for different pretreatment conditions at 0°C. The SFC values at the same temperature were dependent on the holding time at 0°C. The values increased with holding time at 0°C, and this increase was different for different milk fat fractions. The SFC values were similar for 16- and 24-h holding times. After 15°C, the SFC values for different holding times were similar for all fractions.

Milk fat, like most other fats, exhibits polymorphism, which results from a change in crystal structure of the TAG. Crystals of γ, α, β′ and β forms have been identified in milk fat (18–22). The γ form is unstable and has been only observed during photomicrographic studies (19). The α form has little spatial arrangement and a low melting point; β′ crystals have a tighter arrangement and higher melting point; and β crystals have a dense arrangement and the highest melting point (20). The β′ form is generally the most stable form for milk fat crystals (21). The crystallization process is the key force employed for fractionation by MC with or without solvents. Crystal morphology data have been reported in the literature for milk fat fractions. Crystals from MC milk fat fractions exist in β form, whereas crystals from SC-CO_2 fractions exist in β or β′ form (1).

Rapid and deep cooling promotes the formation of the low-melting unstable crystal forms γ and α, but raising the temperature and reducing the cooling rate promote the formation of more stable (β′) crystal forms (19,23–25).

Because the inherent process of milk fat crystallization is relatively slow, the time period allotted for crystallization can affect crystal size and yield. Keogh and Higgins (26) reported an increase in solid fraction yield when crystallization time was increased from 0 to 3 h. Black (27) employed either 16- or 21-h crystallization periods and reported no significant difference in crystal size. Longer crystallization times, though, resulted in increased liquid fat content at separation. Antila (28) noted that 15 h was usually sufficient for crystallization. Many investigators have employed a 24-h crystallization period to ensure that the crystallization process was complete (1). This can also be seen in the data

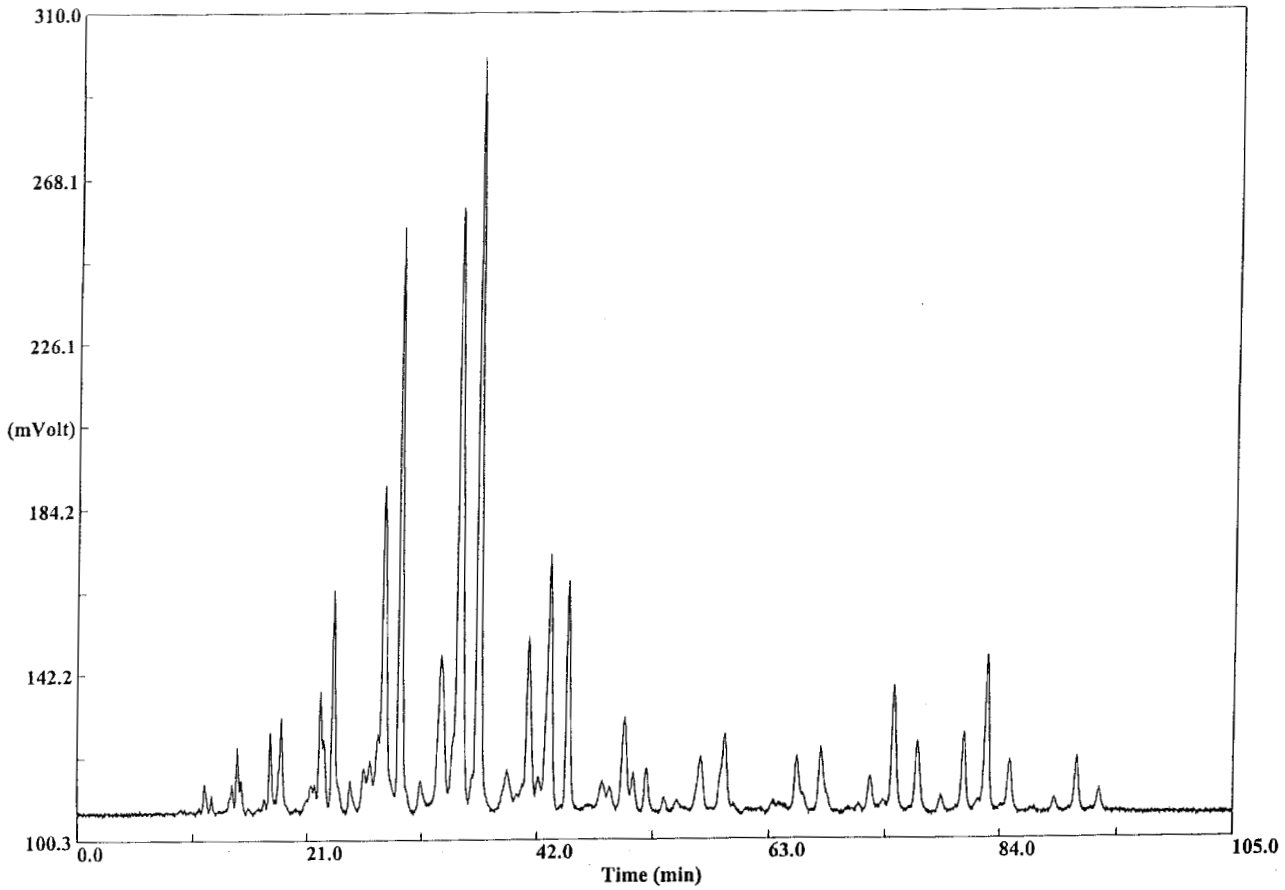

FIG. 5. HPLC profile for SC-CO_2 super olein. See Figures 1 and 3 for abbreviations.

from Table 7 where a holding time of 16 h gave constant SFC values for the fractions.

Because the milk fat fractions have different compositions, a relationship can be seen in NMR data with individual compositions. For example, the AMF10 and super stearin fractions required longer holding times, and both fractions had higher concentrations of unsaturated fatty acids.

At present, there is no commercial plant for SC-CO_2 fractionation of milk fat, whereas MC fractions are available commercially in some European countries. Singh and Rizvi (29) did a detailed economic analysis for continuous SC-CO_2 processing of milk fat, and their results show that SC-CO_2 is economically viable for fractionating milk fat, contrary to what may be the generally held belief. The estimated conversion cost for a 10,000 T/yr SC-CO_2 processing plant was 10–15 cents/kg, compared to 2–5 cents/kg for the MC process. A brief

TABLE 6
Cholesterol and β-Carotene Distribution for Milk Fat Fractions

Fraction	Total carotenoids[a] (μg/100 g)	Cholesterol (mg/100 g)
Milk fat	215	273
Supercritical CO_2		
Super stearin	874	110
Stearin	106	252
Olein	52	307
Super olein	48	345
Melt crystallization[b]		
AMF45	381	217
AMF30	486	302
AMF20	396	443
AMF10	444	454

[a]Measured as β-carotene.
[b]For supplier see Table 3.

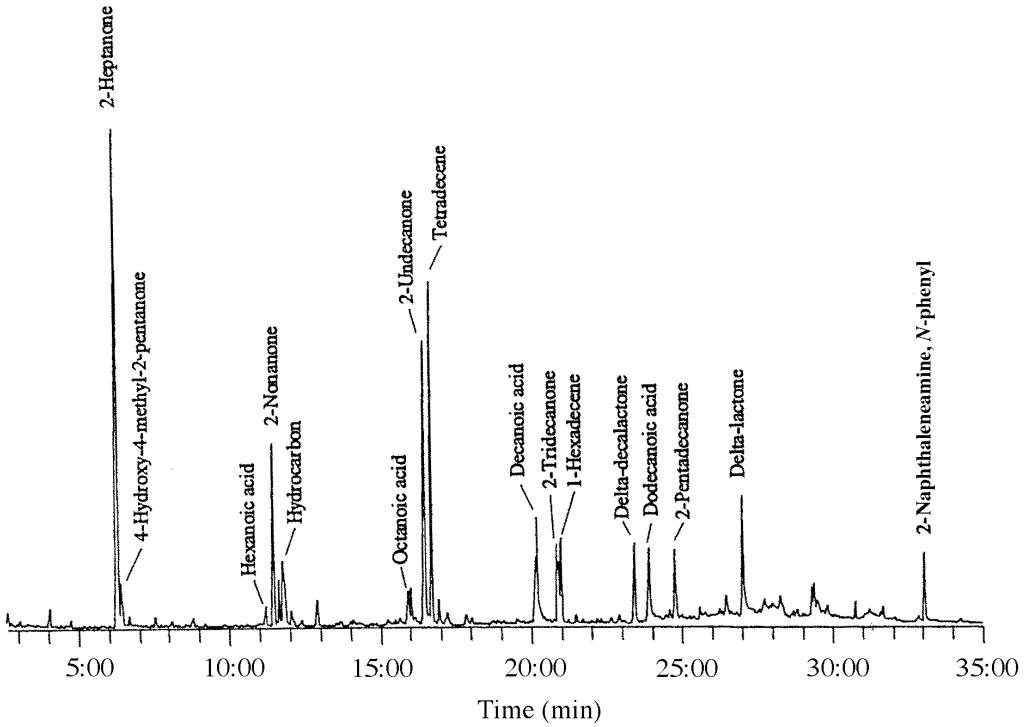

FIG. 6. Volatiles extracted from milk fat.

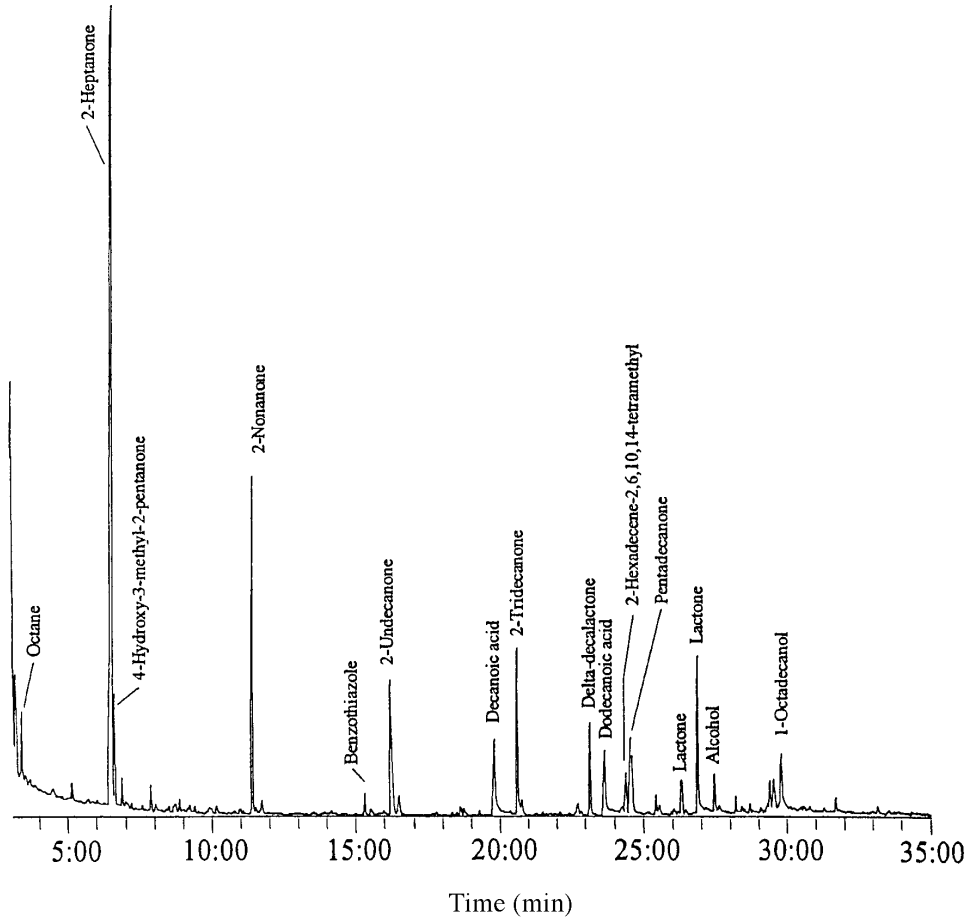

FIG. 7. Volatiles extracted from melt crystallization AMF45. For supplier see Figure 2.

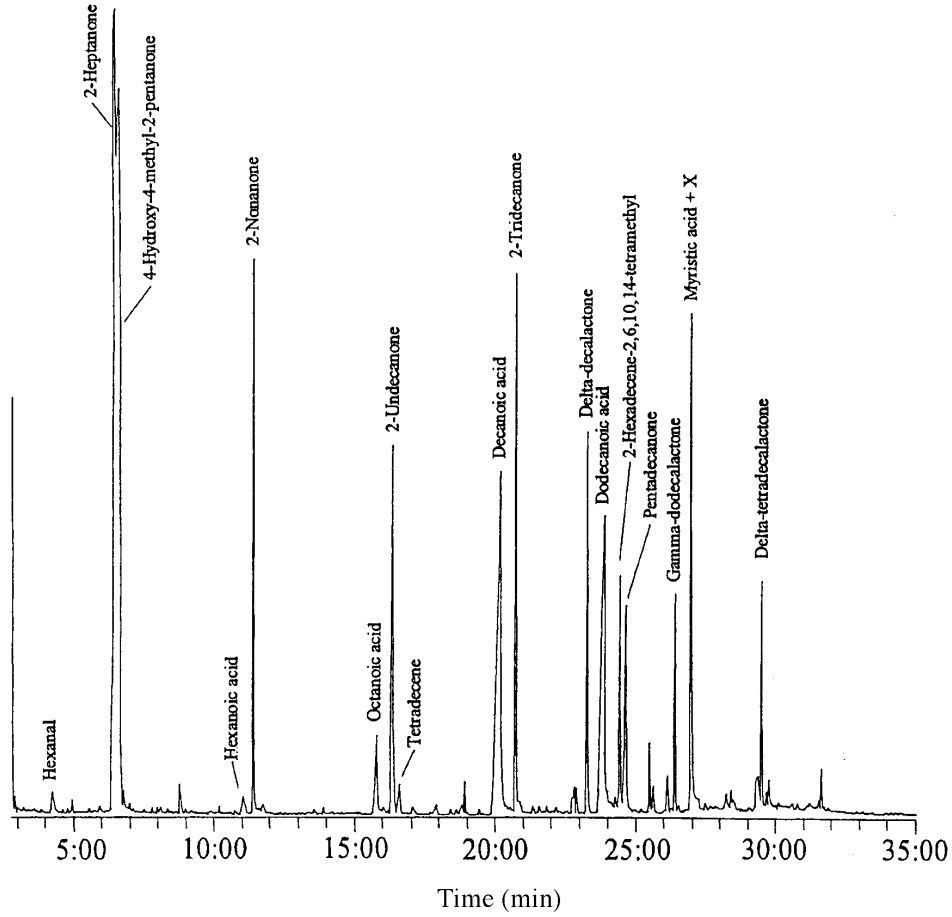

FIG. 8. Volatiles extracted from melt crystallization AMF10.

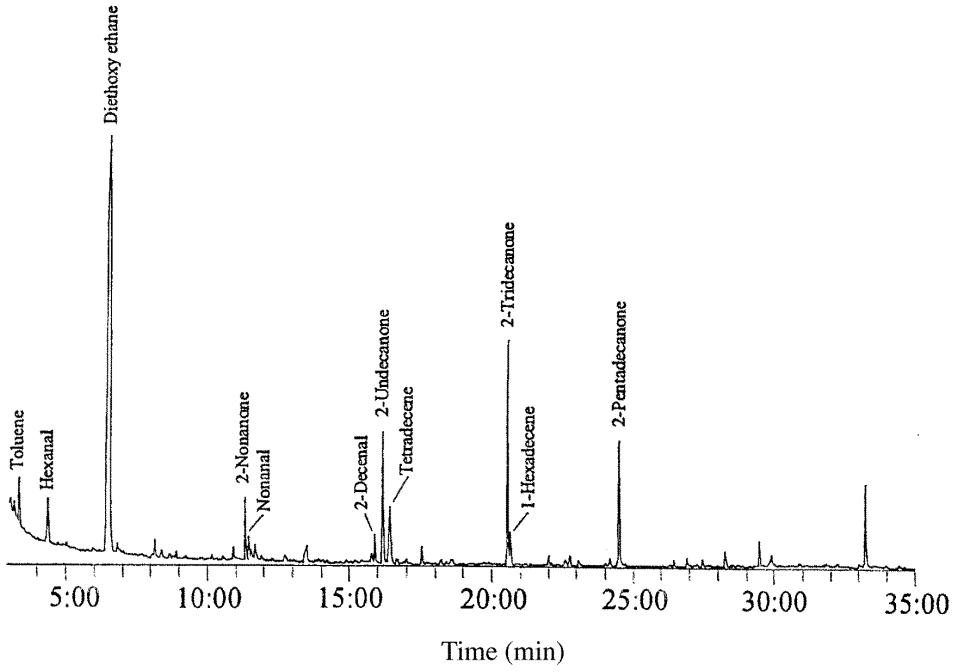

FIG. 9. Volatiles extracted from SC-CO_2 super stearin. See Figure 3 for abbreviation.

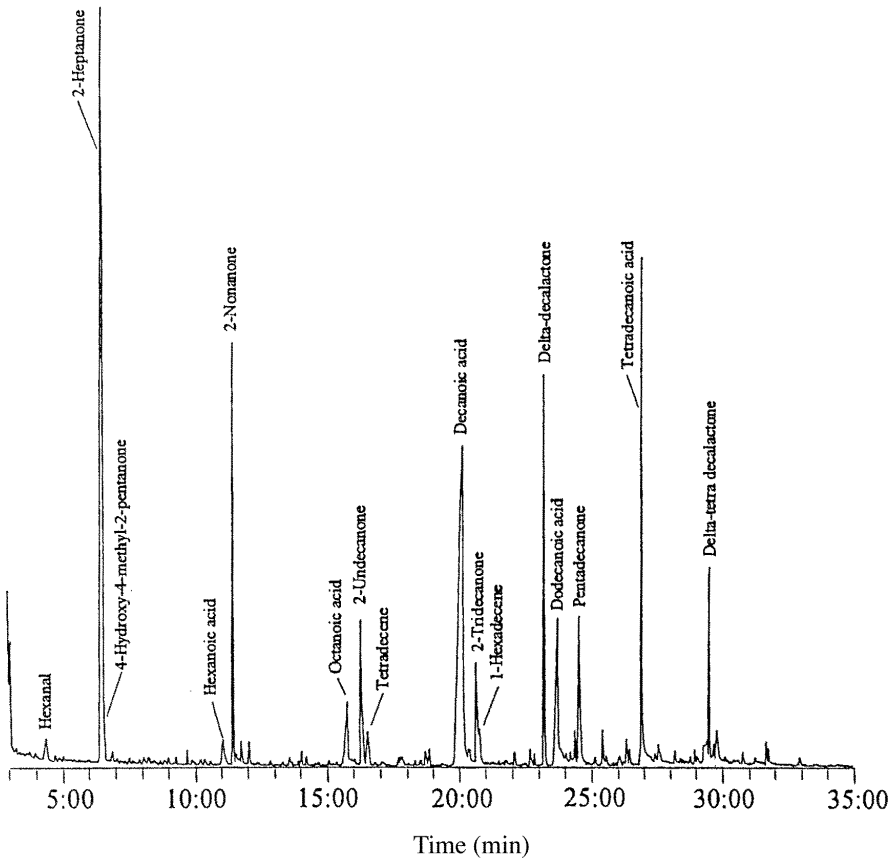

FIG. 10. Volatiles extracted from SC-CO_2 super olein. See Figure 3 for abbreviation.

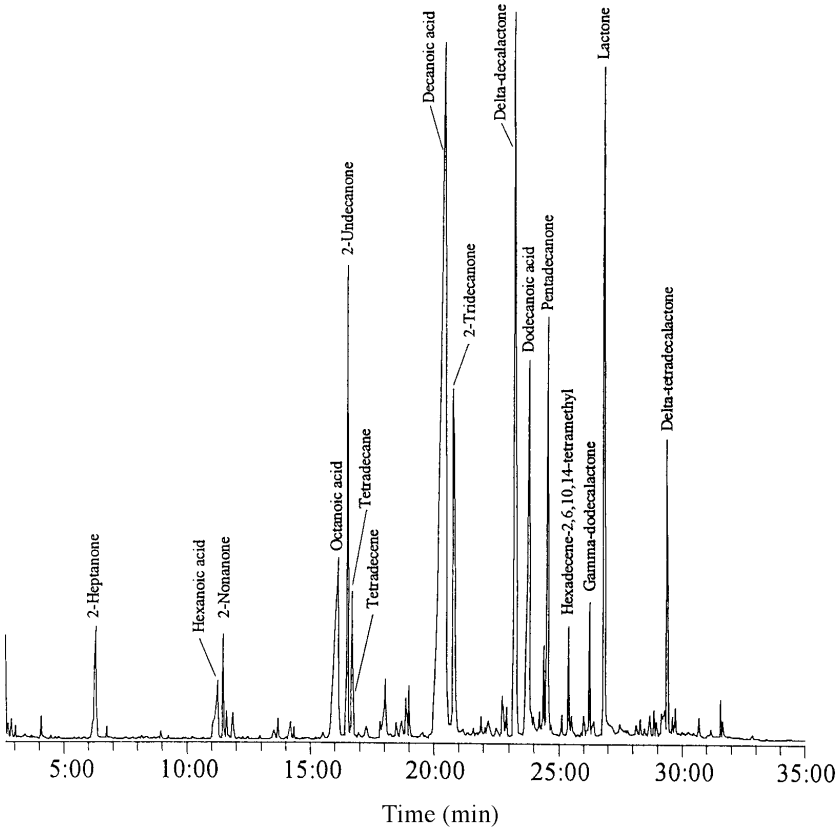

FIG. 11. Volatiles extracted from SC-CO_2 cold trap. See Figure 3 for abbreviation.

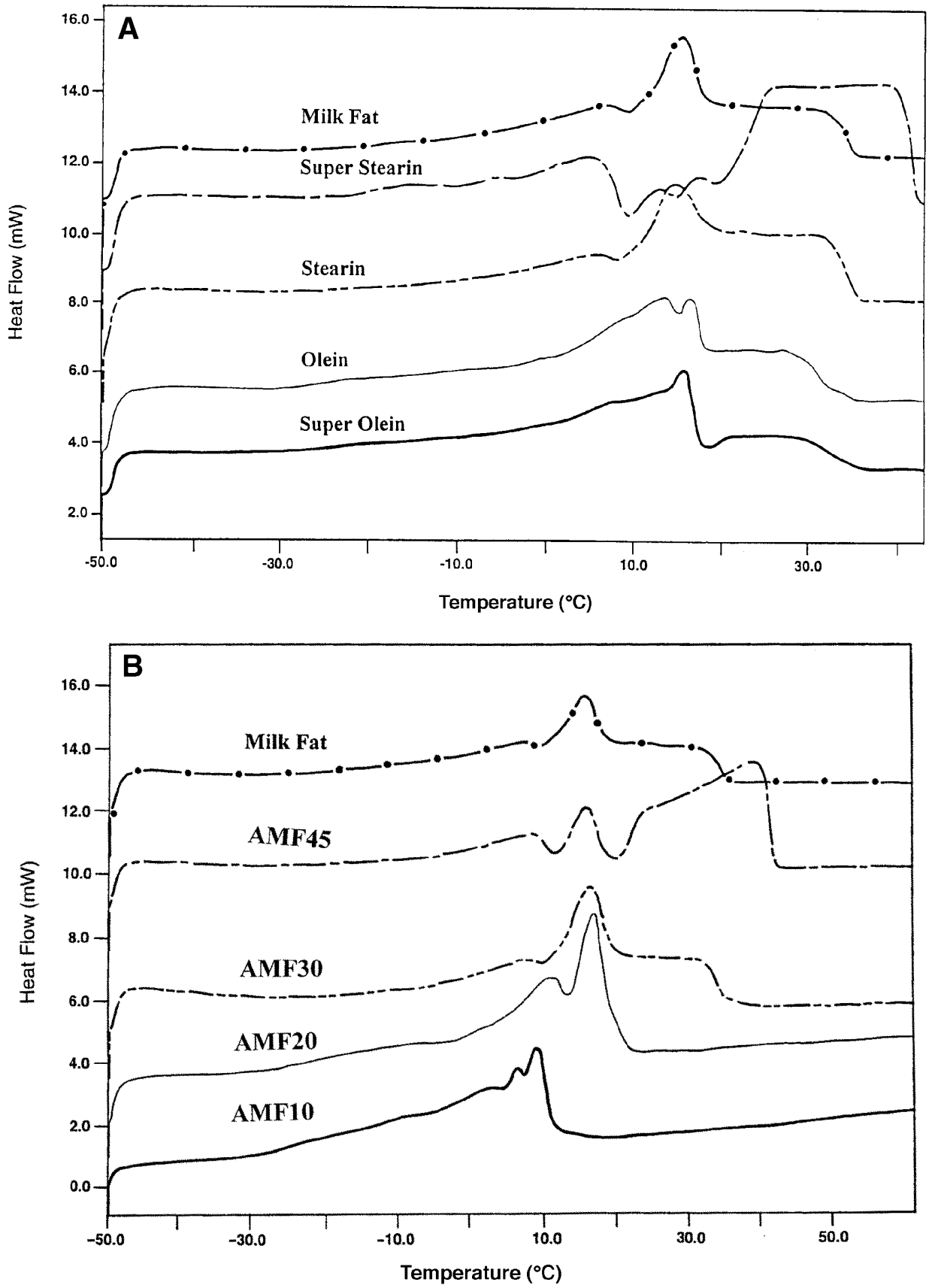

FIG. 12. Differential scanning calorimetry curves for (A) SC-CO_2 milk fat fractions, and (B) melt crystallization milk fat fractions. See Figure 3 for abbreviation.

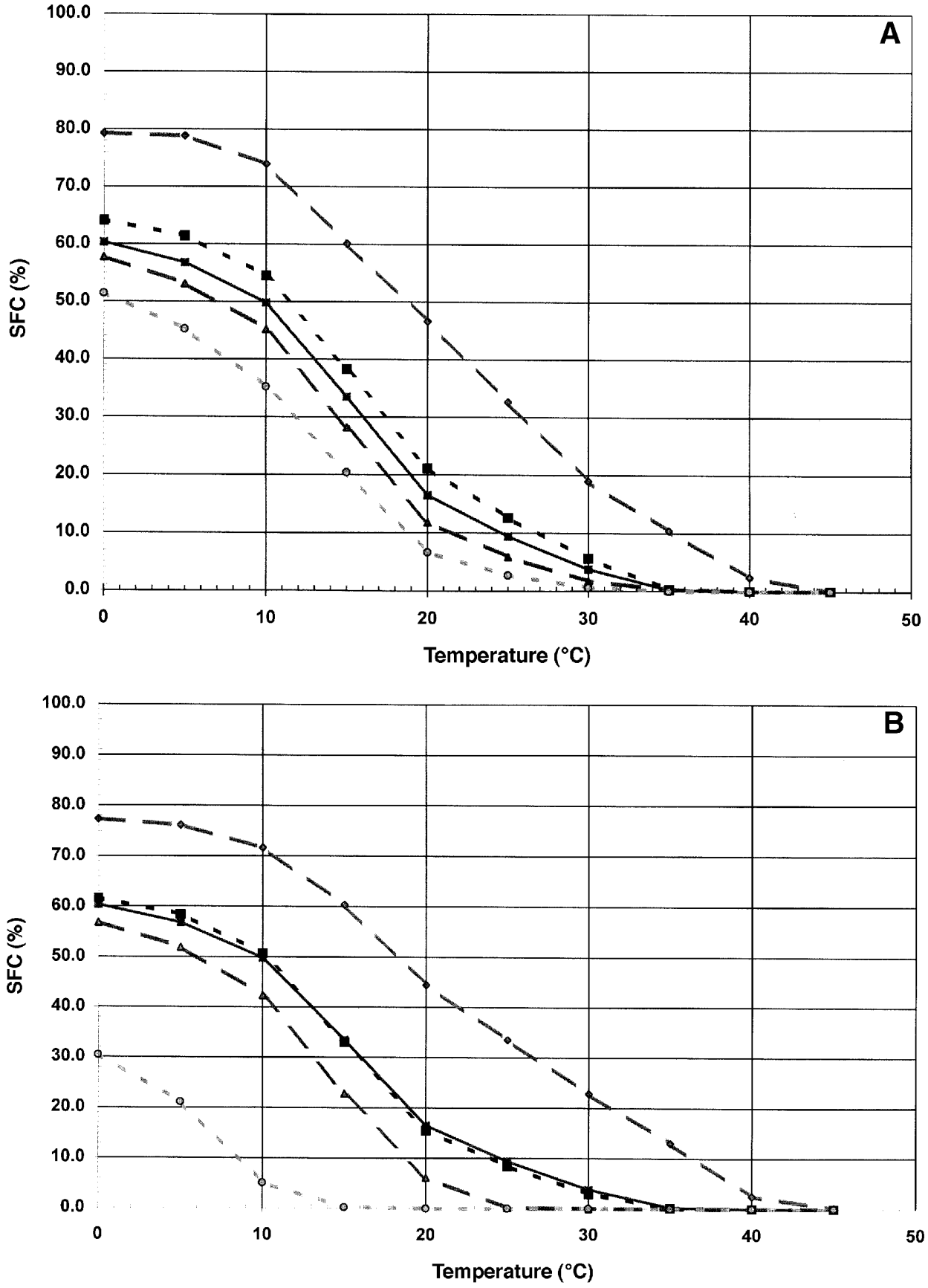

FIG. 13. Solid fat content (SFC) curves for (A) SC-CO_2 milk fat fractions and (B) melt crystallization milk fat fractions. (A) Milk fat (—■—), super stearin (◆), stearin (--■--), olein (▲), super olein (●). (B) milk fat (—■—), AMF45 (◆), AMF30 (--■--), AMF20 (▲), AMF10 (●).

TABLE 7
Solid Fat Content Data Measured by Pulsed Nuclear Magnetic Resonance (NMR) for Different Holding Times at 0°C

Sample	1 h	2 h	3 h	16 h	24 h
Supercritical CO_2					
Milk fat	56.1	58.7	60.4	61.1	61.9
Super stearin	71.0	74.8	76.4	79.8	80.8
Stearin	60.8	63.2	64.0	65.9	65.6
Olein	53.4	56.2	56.9	58.3	58.2
Super olein	47.3	51.0	51.3	51.7	52.4
Melt crystallization[a]					
AMF45	72.5	75.9	76.8	78.5	78.9
AMF30	58.5	61.2	61.5	62.4	60.8
AMF20	52.8	55.5	55.4	57.1	56.9
AMF10	10.2	10.1	18.5	29.7	30.8

[a]For supplier see Table 3.

TABLE 8
Summary of Melt Crystallization and Supercritical CO_2 Fractionation Techniques

	Supercritical CO_2	Melt crystallization
Process	Continuous	Multistage
	CO_2 is nontoxic and low-cost	Simple physical process
	Several fractions per run	Two fractions per run
Fractions	Chemically discrete fractions	Minor differences in composition
High-melting	High unsaturated fatty acids and β-carotene	High saturated fatty acids
Low-melting	High C_4–C_{10} fatty acids	High $C_{18:1}$–$C_{18:3}$ fatty acids
Flavor concentrate	Yes	—
Processing cost	10–15 cents/kg	2–5 cents/kg
Disadvantages	High initial capital investment	Limited temperature range

summary of the advantages and disadvantages of milk fat fractionation methods is presented in Table 8.

In conclusion, it is evident that milk fat fractions offer potential for increasing its utilization. SC-CO_2 and MC produce fractions with different physical properties. However, composition differences are more distinct in the SC-CO_2 fractions. In applications where composition of the fractions is important, SC-CO_2 fractions would have an advantage over MC fractions. With SC-CO_2, the flavor compounds can be simultaneously concentrated with fractionation, i.e., more bland milk fat fractions can be obtained, which could be of interest for special applications, e.g., flavor carriers, filler fats, etc. The solid SC-CO_2 fractions had lower cholesterol and higher β-carotene concentrations, whereas the liquid SC-CO_2 fractions had higher short- and medium-chain fatty acid concentrations. These added advantages in the fractions can help offset some of the higher conversion costs of SC-CO_2 processing.

Applications of other techniques (e.g., interesterification) in combination with fractionation or a combination of MC and SC-CO_2 may further help in targeting the properties of the fractions.

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