Experiment: Milkfat Fractionation

Process Equipment: Supercritical CO_2 Extraction

Material: Milkfat

INTRODUCTION

Milkfat, Anhydrous milkfat, clarified butter or butter oil is one of the major products in the dairy industry and a widely consumed food commodity because of its long shelf-life and contribution to the flavor and quality of the resultant products.

Milk fat has a heterogeneous nature and contains a large variety of fatty acids (approximately 200) with different chain length. Complex fatty acid composition of milk fat results mixture of triglycerides, having different chemical and physical properties. Due to its complex chemical properties, the melting range of milk fat varies from -40 to 40^{o}C. Its origin, season, region of derivation and dependence of feed given to the cows also affect the chemical and physical properties of milk fat.

Table 1. Fatty acid composition of milkfat (g/100 g).

Fatty acid	Milkfat	Name
$C_{4:0}$	2.5	Butyric acid
$C_{6:0}$	1.8	
$C_{8:0}$	1.2	
$C_{10:0}$	2.6	
$C_{12:0}$	3.2	
$C_{14:0}$	10.9	Myristic acid
$C_{15:0}$	1.1	
$C_{16:0}$	28.8	Palmitic acid
$C_{16:1}$	1.4	
$C_{17:0}$	0.6	
$C_{18:0}$	13.3	Stearic acid
$C_{18:1}$	29.2	Oleic acid
$C_{18:2}$	2.7	Linoleic acid
SCFA	5.4	
MCFA	18.7	
LCFA	75.9	

SCFA: short chain fatty acids ($C_{4:0}$- $C_{8:0}$); MCFA: medium chain fatty acids ($C_{10:0}$- $C_{15:0}$)
LCFA: long chain fatty acids ($C_{16:0}$- $C_{18:2}$);

Milk fat has three fractions; the low-, middle-, and high-melting fractions which have distinct chemical and physical properties.

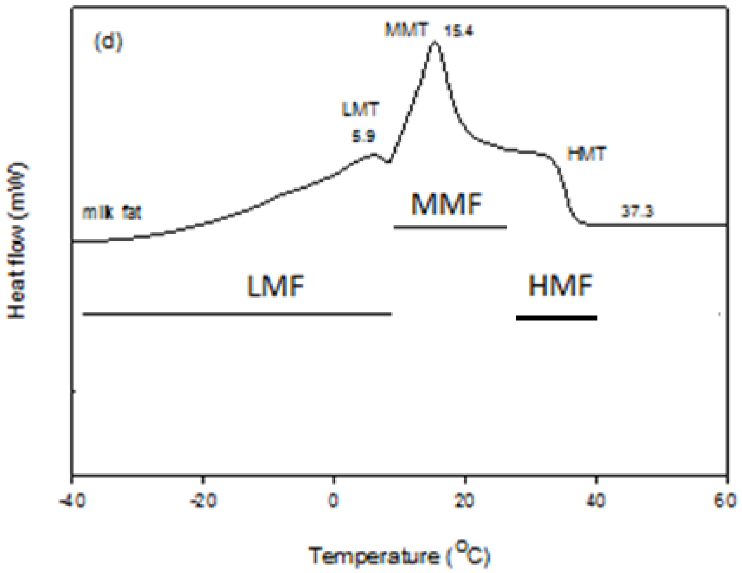


Figure 1. Melting thermogram of milkfat

Each fraction may affect the functionality and behavior in foods together with the other ingredients used in the food product formulations. This behavior varies with the liquid or solid phase, crystal size formed upon rapid or slow cooling from processing temperature and finally its storage. Because of these variations milk fat has some disadvantages. Thus, it is better to use milk fat as fractions depending on the specific desired products, such as low melting in confectionary products, medium melting in biscuits, cakes and pastries and high melting fractions in chocolate and ice cream.

Composition of Oils and Fats

They are composed of Triglycerides

Triglycerides are composed of fatty acids and glycerol

Tristearin: 3*stearic acids melting point around + 71°C

Tripalmitin: 3*palmitic acids melting point around + 60°C

Triolein: 3*oleic acids melting point around - 5°C

Melting Behavior

The melting point of saturated fatty acids increases with increasing chain length

The degree of unsaturation decreases melting point of fatty acids

1. High Melting Triglycerides or Fraction (HMT or HMF)

Long chain saturated fatty acids containing triglycerides **(Palmitic: $C_{16:0}$ and Stearic $C_{18:0}$)**

They melt above room temperature

2. Medium Melting Triglycerides or Fraction (MMT or MMF)

Medium chain saturated fatty acids containing triglycerides **($C_{10:0}$, $C_{12:0}$, $C_{14:0}$)**

They melt between $0^{o}C$ - room temperature depending on the linear chain length

3. Low Melting Triglycerides or Fraction (LMT or LMF)

Short chain saturated fatty acids containing triglycerides + Long chain unsaturated fatty acids containing triglycerides (**$C_{4:0}$, $C_{6:0}$, $C_{8:0}$ and $C_{18:1}$ and $C_{18:2}$**)

They melt below $0^{o}C$

Fractionation technology of milk fat has been developed to use milk fat efficiently in food products. These methods must be applied in batch or continuous systems to fractionate milkfat into fractions.

Fractionation Methods of Milkfat

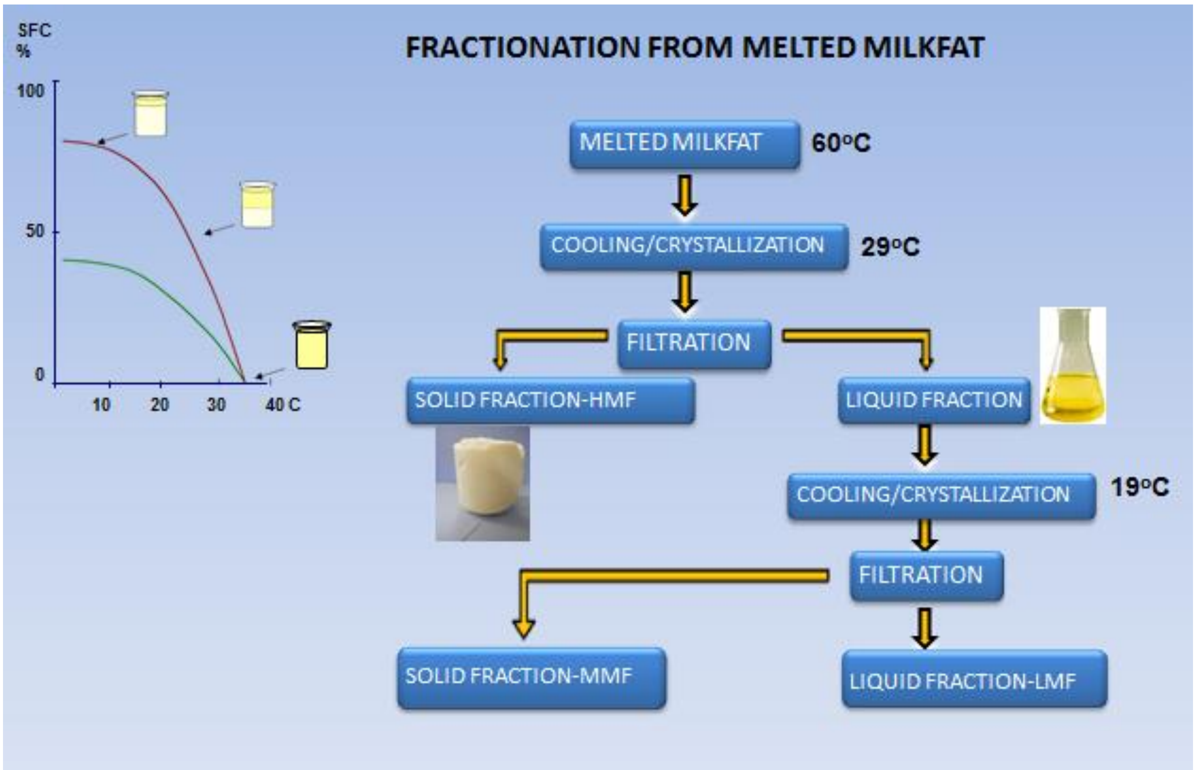
a- Crystallization from Melted Milkfat (dry fractionation)

b- Crystallization using Solvents from Melted Milkfat

c- Short-Path Distillation

d- Supercritical Fluid Extraction

a,b- Crystallization from melted milkfat and using solvent



Dry and solvent fractionation involve the crystallization of triglycerides based on melting behavior of milkfat.

First crystallized solid fraction at 29°C has high amount of long chain saturated fatty acids (stearic and palmitic) containing triglycerides (HMF).

Second crystallized solid fraction at 19°C has high amount of medium chain saturated fatty acids ($C_{10:0}$- $C_{15:0}$) containing triglycerides (MMF)

Remaining fraction has high amount of short chain saturated ($C_{4:0}$- $C_{8:0}$) and long chain unsaturated fatty acids (oleic and linoleic) containing triglycerides (LMF)

c. Short Path distillation fractionates milkfat according to molecular weight of triglycerides

Short-path distillation permits separation of fats based on triglyceride boiling points under vacuum, can result in distillates enriched in volatile and low molecular weight components or higher molecular weight triglycerides depending on the temperature and applied vacuum used. The controlling factor is the rate at which the molecules escape from the heated surface of the distilling liquid and are received by the cooled condenser surface. Contrary to other techniques, in short-path distillation there is a gradual increase in concentration of unsaturated long-chain fatty acids in the solid fraction

Short-path distillation effects a very high degree of molecular weight separation, which offers an excellent opportunity to obtain fractions with distinctive chemical and physical properties.

First fraction (boiled and condensed) has high amount of short chain saturated fatty acids containing triglycerides (low molecular weight triglycerides or fraction-LMWF).

Second fraction (boiled and condensed) has high amount of medium chain saturated fatty acids containing triglycerides (medium molecular weight triglycerides or fraction-MMWF).

Third fraction (boiled and condensed) has high amount of long chain saturated and unsaturated fatty acids containing triglycerides (high molecular weight triglycerides or fraction-HMWF).

d. Supercritical Fluid Extraction fractionates milkfat according to molecular weight of triglycerides

Supercritical fluid extraction (SFE) is a separation process where the properties of a solvent in a supercritical state can selectively extract specific compounds or to fractionate mixtures based on solvent temperature and pressure.

Table 2. Critical conditions for various supercritical solvents.

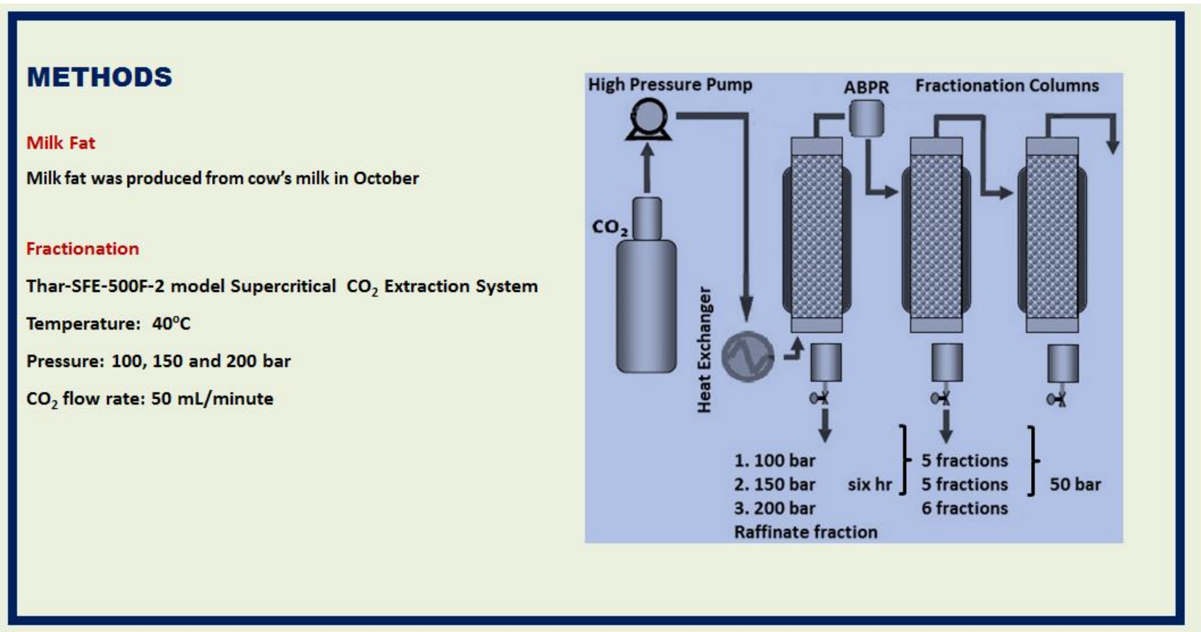
Fluid	Critical Temperature (K)	Critical Pressure (bar)
Carbon dioxide	**>304.1**	**> 73.8**
Ethane	>305.4	>48.8
Trifluoromethane	>299.3	>48.6
Chlorotrifluoromethane	>302.0	>38.7
Trichlorofluoromethane	>471.2	>44.1
Ammonia	>405.5	>113.5
Water	**>647.3**	**>221.2**
Cyclohexane	>553.5	>40.7
n-Pentane	>469.7	>33.7
Toluene	>591.8	>41.0

Supercritical carbon dioxide (SC-CO_2) is as an attractive alternative to conventional extraction using organic solvents given increasing public awareness of the health, environmental and safety hazards associated with organic solvents in food processing and possible solvent contamination. It has been used for the extraction and fractionation of lipid-bearing materials, decaffeination and the extraction of cholesterol, aromas and antioxidants from fats and oils.

Beside CO_2, water is the other increasingly applied solvent. One of the unique properties of water is that, above its critical point, it becomes an excellent solvent for organic compounds and a very poor solvent for inorganic salts. However, water has negative effect on oils due to high temperature for supercritical region. This fractionation technique increases the solubility of triglyceride in the supercritical region of CO_2 by adjusting the CO_2 pressure and temperature. Hence milk fat can be fractionated into different molecular weight fractions by using the SC-CO_2 method. Products are enriched and separated into short-chain, medium-chain and long-chain triglycerides.

FRACTION METHOD

Fractionation was carried out using a laboratory-scale SC-CO_2 system (Thar Model SFE-500F-2, Thar Technologies Inc., Pittsburgh, PA, USA) that consisted of a high pressure CO_2 pump, heat exchanger, automatic back pressure regulator (ABPR), three 500 cm^3 stainless-steel vessels (raffinate, first and second fractionation columns).



Approximately 100 g of melted milkfat was placed into the raffinate vessel for each process. The fractionation schemes performed in this study are presented below. Three sequential processes were followed for the fractions using same milk fat.

Process	Pressure (bar)	Process time (hr)	Number of sample
Run 1	100 bar (10 MPa)	6	5
Run 2	150 bar (15 MPa)	6	5
Run 3	200 bar (20 MPa)	6	6

The fractions were continuously collected every 1 h for 6 h at each pressure. For run 1, the pressure inside the raffinate vessel was maintained at 90 bar for 30 min to allow sufficient contact of the milkfat with the supercritical CO_2. It was then kept at 100 bar for the first fractionation run by using an automated back pressure regulator. Afterwards, the pressure in the first fractionation vessel was adjusted to 50 bar, at which point a valve between the first and second vessels was opened along with a vent to keep the pressure and CO_2 flow constant.

Fractions were collected hourly from the bottom of the first vessel at the operational pressure. The procedure was repeated with pressures of 150 and 200 bar. Each run was completed in 6 h at a 50 mL/min CO_2 flow rate. In all, sixteen fractions plus raffinate were collected at 40°C from the same starting batch sample. Rate of fractionation, fatty acid composition and melting behavior were determined for fractions.

RESULTS and DISCUSSION

Rate of Fractionation

The fractionation yields at 10, 15 and 20 MPa were 34, 26 and 33 g/100 g milkfat, respectively (Figure 2). The weight of the raffinate (the high-molecular weight) was 7 g/100 g milkfat. At 10 MPa, the extent of fractionation initially increased with time and then decreased after 3 h as the lower melting species soluble became depleted. The extraction rate at 15 MPa was lower than at 10 MPa, given the apparent lack of soluble components at this pressure. Fractionation at 20 MPa was linear over 6 h and showed a yield similar to 10 MPa. At room temperature, all 10 MPa fractions were liquid whereas those collected at 15

MPa were semi-solid and those at 20 MPa were solid. All fractions were colourless, except for the yellowish raffinate and 20 MPa-6 h fraction dueto presence of β-carotene.

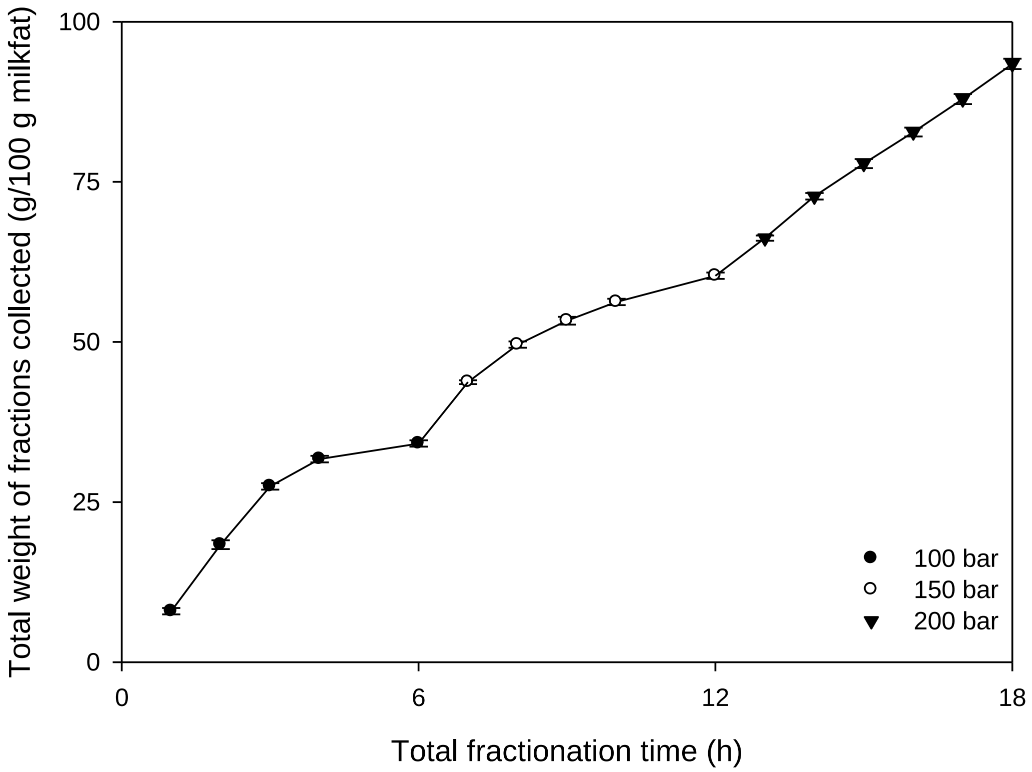


Figure 2. Total weights of milkfat fractions at the different fractionation pressures.

Fatty acid composition (Table 2)

The fractions collected at 10 MPa contained triglycerides enriched in short chain fatty acids whereas at 15 MPa, there was no preference in fatty acid type extracted. The 20 MPa fractions and raffinate were richest in long chain fatty acids, namely stearic, oleic and linoleic acids.

With pressure, the concentration of short chain (C_4–C_8) and medium chain (C_{10}–C_{15}) fatty acids decreased whereas the long chain (C_{16}-$C_{18:2}$) and total unsaturated fatty acids increased. The ratio of all unsaturated to saturated fatty acids increased from 10 to 20 MPa, ranging from 0.28 to 0.81 as compared to a value of 0.52 for milkfat. Thus, changes in pressure yielded fractions markedly different composition.

Table 2. Fatty acid composition of milkfat and its fractions (g/100 g).

Fatty acid	Milkfat	100 bar					150 bar					200 bar						Raffinate
		1 h	2 h	3 h	4 h	6 h	1 h	2 h	3 h	4 h	6 h	1 h	2 h	3 h	4 h	5 h	6 h	
$C_{4:0}$	2.5	6.1	5.9	4.9	4.6	4.5	3.3	2.7	2.3	2.1	1.8	1.1	0.7	0.4	0.2	0.2	-	-
$C_{6:0}$	1.8	3.7	3.5	3.1	3.0	3.0	2.5	2.3	2.1	2.0	1.7	1.2	0.9	0.6	0.4	0.3	0.2	0.2
$C_{8:0}$	1.2	2.5	2.1	1.7	1.6	1.6	1.5	1.5	1.4	1.4	1.3	1.0	0.8	0.6	0.4	0.3	0.2	0.2
$C_{10:0}$	2.6	5.0	4.2	3.4	3.2	3.2	3.2	3.2	3.2	3.2	3.1	2.5	2.3	1.9	1.5	1.2	0.9	0.7
$C_{12:0}$	3.2	5.6	4.9	4.0	3.7	3.6	3.4	3.4	3.4	3.4	3.4	3.0	2.9	2.6	2.3	2.0	1.6	1.4
$C_{14:0}$	10.9	15.3	14.7	13.2	12.7	12.6	11.2	11.0	10.9	10.9	10.9	10.3	10.1	9.8	9.4	8.8	7.9	7.3
$C_{15:0}$	1.1	1.3	1.3	1.2	1.2	1.2	1.1	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	0.9
$C_{16:0}$	28.8	30.3	31.5	31.7	31.7	32.1	30.4	29.9	29.5	29.3	28.9	27.8	27.5	27.1	26.8	26.3	25.9	26.3
$C_{16:1}$	1.4	0.6	0.6	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.8	0.8	0.7
$C_{17:0}$	0.6	0.5	0.5	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.7	0.7
$C_{18:0}$	13.3	8.1	8.6	10.0	10.4	10.7	12.0	12.5	12.8	13.1	13.3	14.1	14.5	15.0	15.5	15.7	17.0	19.1
$C_{18:1}$	29.2	18.1	19.2	22.4	23.2	22.9	26.6	27.6	28.4	28.6	29.4	32.7	33.9	35.5	36.9	38.6	39.9	38.8
$C_{18:2}$	2.7	1.9	2.1	2.3	2.4	2.4	2.6	2.7	2.7	2.8	2.8	3.0	3.1	3.1	3.2	3.3	3.2	3.0
SCFA	5.4	12.3	11.5	9.6	9.1	9.1	7.3	6.5	5.8	5.5	4.8	3.2	2.4	1.6	1.0	0.8	0.6	0.5
MCFA	18.7	28.3	26.1	22.8	21.9	21.6	19.8	19.6	19.5	19.6	19.5	17.9	17.3	16.4	15.2	13.9	12.0	11.0
LCFA	75.9	59.4	62.5	67.6	69.0	69.3	72.9	73.9	74.7	75.0	75.7	78.9	80.3	82.0	83.8	85.3	87.4	88.6
USFA	34.2	21.6	22.9	26.3	27.2	26.9	30.9	32.0	32.8	33.1	34.0	37.5	38.8	40.4	41.8	43.5	44.6	43.1
SFA	65.8	78.4	77.1	73.7	72.8	73.1	69.1	68.0	67.2	66.9	66.0	62.5	61.2	59.6	58.2	56.4	55.4	56.9

SCFA: short chain fatty acids ($C_{4:0}$- $C_{8:0}$)

MCFA: medium chain fatty acids ($C_{10:0}$- $C_{15:0}$)

LCFA: long chain fatty acids ($C_{16:0}$- $C_{18:2}$)

USFA: unsaturated Fatty Acids, SFA: saturated Fatty Acids

Thermal behaviour

Melting thermograms of milkfat and fractions (Figures 3) agreed with previous studies. The melting thermogram of milkfat exhibited three endotherms representing the low- (LMT), medium- (MMT) and high-melting (HMT) Triglycerides.

At 10 MPa, only the LMT and MMT were initially extracted with the HMT appearing after 3 h (Figure 3a). At 15 MPa, the HMT became very pronounced such that by 6 h of fractionation, it was nearly equal in intensity to the MMT (Figure 3b). At 20 MPa, the HMT dominated all melting thermograms (Figure 3c). The raffinate consisted of mostly higher-melting Triglycerides along with small amounts of low and middle-melting Triglycerides (Figure 3d). The raffinate showed a high-melting fraction at 20 °C. The melting thermogram of milkfat was similar to the 20 MPa-1 hr fraction.

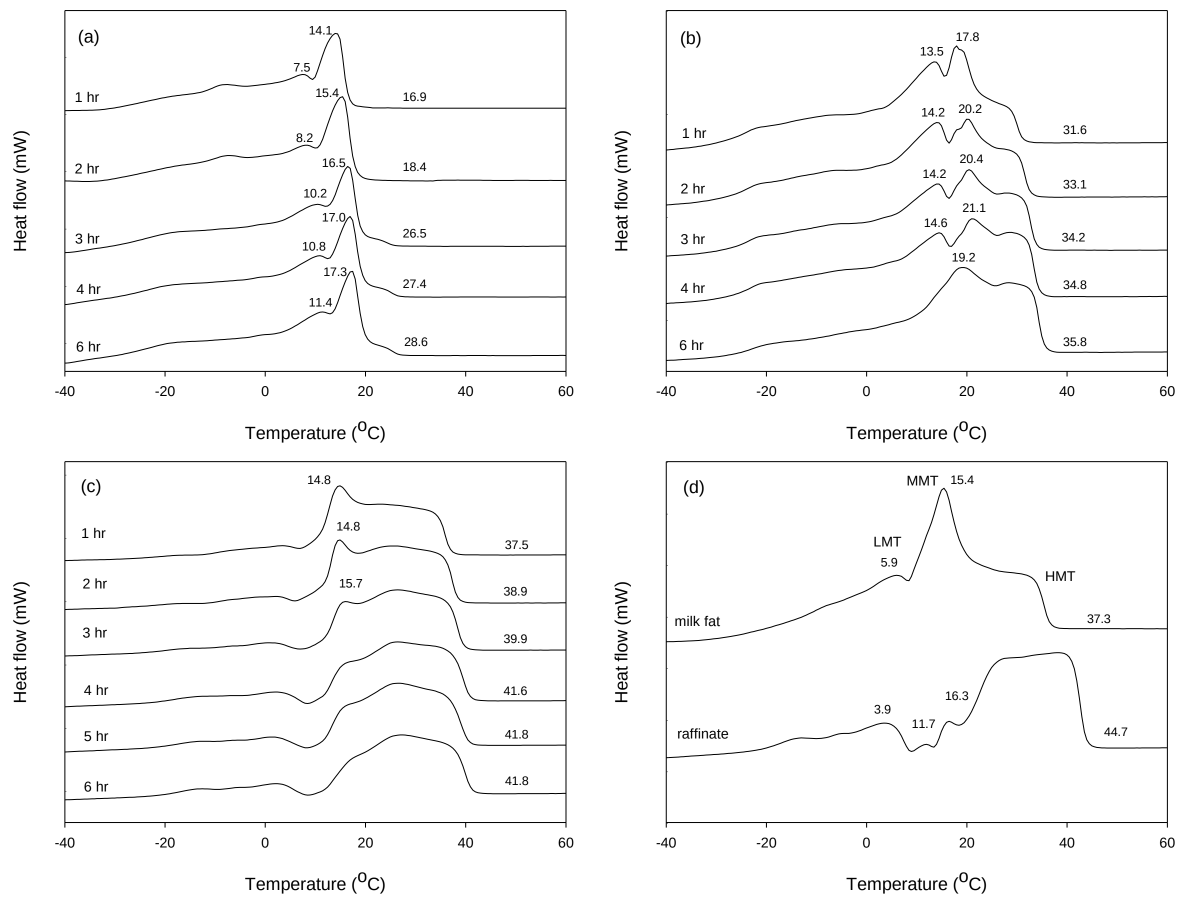


Figure 3. Melting thermograms of fractions produced at (a) 100, (b) 150, (c) 200 bar, and (d) milkfat and raffinate.