Effect of Melting Point on the Physical Properties of Anhydrous Milk Fat

Yunna Wang[1], Yang Li[1], Jie Han[1], Yan Li[2], Liebing Zhang[1,*]

[1]College of Food Science and Nutritional Engineering, China Agricultural University, No.17, Qinghuadonglu, Haidian District, Beijing 100083, China
[2]Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology and Business University, Beijing 100048, China

*Corresponding author e-mail: liebzhang@gmail.com

Abstract. The effect of melting point on the physical properties of anhydrous milk fat were investigated. The results showed that high melting fractions (HMF) (S30,S35) were enriched in long-chain fatty acids, whereas low melting fractions (LMF)(S5,S10,S15) were enriched in short-chain and unsaturated fatty acids. From S5 to S35, enthalpy value was gradually increased on both crystallization and melting condition, so as SFC on different temperature. The mixture and chemical interesterification allowed obtaining fats with various degrees of plasticity, increasing the possibilities for the commercial use of different fraction of AMF.

1. Introduction

Milk fat (MF) has about 400 types of fatty acids which differ in chain length (C4 to C18), and a wide range of molecularweight species of triacylglycerols (from 28 to 56 carbon atoms) [1]. Moreover, MF is a relatively high saturated fat: about 65% saturated fatty acids (mainly C16:0, C18:0 and C14:0) and about 35% unsaturated fatty acids (mainly C18:1). Therefore, MF melts within a wide temperature range (−40 to 40 °C). Anhydrous MF (AMF) has the potential to be used as a trans-fatty acids replacement due to its low content in palmitic and trans-fatty acids [2]. Besides fat composition, AMF has good sensory attributes such as flavor and mouthfeel.

The most essential physical properties of AMF are crystallization and melting behavior because these properties affect the functional and sensory attributes of AMF-containing products (i.e. firmness and spreadability of butter, structure of whipped cream, glossiness and mouth feel of chocolate and smoothness of ice-cream) [3, 4]. The most common methods to study crystallisation of fats are differential scanning calorimetry (DSC) and pulsed nuclear magnetic resonance (pNMR). These methods give information about the thermodynamic of the primary crystallisation, but not about the development of structure. Since it was found that the SFC content of fat measured at different temperatures can be used as a prognostic indicator of the important attributes of products, such as chewing feeling and hardness. Kinetics measurements of SFC can provide insight into the mechanism of crystallization of the fat mixture which directly causes the quality of the final product. SFC-profile may be useful information for creating the physical properties of the product and predicting the behavior of the final product during storage and transport [3]. pNMR is the only method that directly

measures the SFC as opposed to indirect methods of DSC where the results are obtained by measuring the heat capacity as for volume changes resulted from the melting of the solid phase.

The objective of this study was to characterize the chemical composition of different melting fraction obtained by dry fractionation from the same batch of AMF, and to investigate the effects of AMF at various melting fractions on the physicochemical composition, crystallization and thermal properties. Results will strengthen the understanding of interactions between high-melting fat and various low-melting fat. This information will be useful during the application of such blends in the food.

2. Materials and methods

2.1. Samples

Anhydrous MF (AMF) was purchased from Fonterra Cooperative Group (Auckland, New Zealand). According to the manufacturer, the AMF contained 99.9% butter fat, less than 0.1% moisture, 0.2% free fatty acid (expressed as oleic acid). The AMF was stored at 4°C upon arrival. AMF is comprised of a wide range of different triacylgly-cerols, containing the widest range of both saturated and unsaturated fatty acids (from C4:0 to C18:3) of any known natural fat. Three different components, based on melting point, are generally recognized [5].

2.2. Analysis of fatty acid composition

Gas chromatography-flame ionization detector (GC-FID) was used to analyse gross fatty acid compositions of AMF as Fatty Acid Methyl Esters (FAMEs), as described by [6]. Briefly heptanoic acid (1 mg) was used as internal standard. This internal standard and methanolic NaOH (0.5 M; 0.5 mL) were added to lipid sample (15-20 mg). Saponification process was undertaken by placing the lipid sample, which was previously flushed with nitrogen gas, on a steam bath at 95 °C for 3 min. After cooling the sample, esterification of fatty acid was performed by adding a catalyst (2.5 mL of boron trifluoride (BF3) in methanol (14%, v/v)) to the lipid sample, followed by heating on the steam bath for 5 min and then allowed to cool. Addition of heptane (2 mL) and vortex were undertaken. After allowing the two layers to settle, clear heptane solution (1.5 mL) was transferred into the auto sampler vial. All samples were analyzed in duplicate and the fatty acid contents are presented as mean±standard deviation in Table 1.

2.3. Thermol behavior

The non-isothermal crystallization conditions were as follow: The thermal behavior of the AMF melting fractions were determined by differential scanning calorimetry (DSC), Model Q2000 (TA Instruments, USA) according to the method AOCS Cj 1–94 (2004). Approximately 10 mg of sample were weighed into aluminum pans hermetically closed, and subjected to initial temperature of 40 °C with subsequent cooling rate of 5°C min^{-1} to -10°C[7].

The isothermal crystallization conditions were as follows: The crystallization isotherms were determined at 4°C, by increasing the solids content as a function of time by NMR and reading chamber stabilized at 4°C [8]. The crystallization parameters, including the induction time (min) and maximum solids content (%) were obtained from the isotherm after 120 min. The analysis was performed in duplicate.

2.4. Solid fat content

The solid fat content (SFC) was determined in a nuclear magnetic resonance spectrometer (NMR) (nuclear magnetic resonance) (Bruker Minispec PC120, Germany), according to the AOCS Cd 16b-93 methodology (2009). About 5 mL of samples were first heated for 15 min at 80°C, kept for 5 min, and stabilized at 0°C for 1 h using a high-precision dry bath (TCON 2000 Duratech, USA). Then 2 mL of melted fat was poured into a pNMR glass tube. The tube was then placed in the pNMR in series at temperatures of 5, 10, 15, 20, 25, 30, 35 and 40 °C [8]. The analysis was performed in duplicate.

3. Results and Discussion

MF fractionation is an industrial process employed to obtain butters or dairy products with specifically adapted thermal properties. This study analyses the relation between fraction compositions of MF industrially fractionated at different melting fractions (35°C, 30°C, 25°C, 20°C, 15°C, 10°C, 5°C) and their thermal and structural properties.

3.1. Fatty acid composition of the different melting fractions

The gross fatty acid compositions of the different melting fractions are presented in Table 1. All melting fractions were enriched with saturated fatty acids (90±5%), in which stearic acid (35±5%) was predominating. In comparison with S5, distinct differences in compositions of S35 can be observed. Obviously, the saturated fatty acids content (95.84%) in S35 was higher, while unsaturated fatty acids content in S35 (4.16%) was lower than those in S5~S30 (13.17%&12.83% &12.35&10.54% &8.44% &5.39, respectively). This result indicates that the step-wise dry fractionation of melted AMF was able to separate AMF with distinct chemical composition. This result was similar to the one previously reported [9].

The analysis of chemical composition of S5 and S10 showed that both of them mostly consisted of unsaturated fatty acids (13.17%&12.83%, respectively). Among unsaturated fatty acids, oleic and linoleic were highly concentrated in S5, S10, S15, while Stearic acid (39.06%) were rich in S35. It appears that all melting fractions had overall similarity in terms of distribution and concentration of fatty acid components (Table 1). Thus, the molecular compatibilities between S5 and S35 investigated may be different, resulting in discrepancies in physical and thermal properties. For example, greater resemblance in chemical structure between the fatty acids of the mixture can ease the formation of compound crystals due to their high miscibility in solid state [10].

Table 1. Fatty acid composition of different melting fractions (S35, S30, S25, S20, S15, S10, S5)

Fatty acid common name	Weight (%)						
	S5	S10	S15	S20	S25	S30	S35
Saturated							
Butyrate	1.32	1.19	1.07	0.76	0.32	0.3	0.23
Hexylic	1.86	1.73	1.68	1.7	0.68	0.43	0.22
Caprylic	2.91	2.79	2.73	1.91	0.83	0.58	0.45
Capric	2.8	2.38	2.29	2.45	2.71	2.18	2.07
Lauric	3.63	3.68	3.95	4.62	4.45	4.04	4.13
Myristic	11.31	11.47	11.32	11.53	11.99	13.7	13.79
Pentadecanoic	1.43	1.56	1.78	1.69	2.43	2.45	2.57
Palmitic	25.74	26.61	26.47	26.64	27.75	29.65	30.54
Heptadecanoic	1.11	1.67	1.71	2.29	2.64	2.72	2.78
Stearic	34.72	34.09	34.65	35.87	37.76	38.55	39.06
Sum of saturated FAs	86.83	87.17	87.65	89.46	91.56	94.6	95.84
Unsaturated							
Hexadecenoic	3.97	3.85	3.41	3.37	2.11	1.42	0.72
Oleic	3.65	3.79	3.49	2.35	2.17	0.45	0.74
Linoleic	3.74	3.31	3.86	2.95	3.43	2.85	2.22
Linolenic	1.81	1.88	1.59	1.87	0.73	0.67	0.48
Sum of unsaturated FAs	13.17	12.83	12.35	10.54	8.44	5.39	4.16

Reported values were means of two replicates (standard deviation).

3.2. Thermal properties of the different melting fractions

Both of two main exothermic peaks in S35 and S30 shifted significantly (P < 0.05) to higher temperatures in comparison to those of S25 and S20, and the first higher-temperature exotherm in the cooling curve of S35 and S30 became much greater. These results can be attributed to the greater proportion long-chain fatty acids (palmitic and stearic acids) in S35 and S30 (Table 1). Increased proportion of high melting point fractions shift to higher crystallization temperature is caused by the inter-solubility as high melting point fractions are normally solubilised in the low melting liquid fractions [11; 12]. Enthalpies and peak temperatures of representative transitions extracted from DSC thermograms are expressed as mean ± standard deviation and tabulated in Table 2. First crystallization peak of S15, S10, S5 were not exist, and the small shoulder peak became less distinct. The crystallization enthalpy changed significantly from S5 and S35 (Table 2).

Both crystallization and melting, enthalpy value of S5 to S35 were gradually increased (Table 2). Apart from transition temperature, changes in enthalpy values reflect differences in the type, compactness, purity and/or amount of crystals (polymorphs) formed. It has been reported that an increase in melting enthalpy (without changing of SFC) was associated with formation of larger and higher-melting crystal form in margarine [13]. Shen et al. [14] also pointed out that the total melting enthalpy of AMF/hydrogenated coconut oil blends increased with increasing amounts of hydrogenated coconut oil. It has also been reported that melting and crystallization enthalpies of the refined hoki oils were related to their fatty acid contents. The enthalpy values were lower for the oils that have more polyunsaturated fatty acids [15].

The DSC thermo grams of seven different meltiong fractions indicate that the step-wise dry fractionation of molten AMF was able to separate these fractions with distinct thermal properties.

Table 2 Peak temperatures of representative transitions and total enthalpies of different melting fractions and their mixtures during cooling and heating processes

Composition	Crystallization				Melting			
	T_{1C} °C	T_{2C} °C	T_{3C} °C	$\triangle H_{C, Total}$ J g^{-1}	T_{1M} °C	T_{2M} °C	T_{3M} °C	$\triangle H_{M, Total}$ J g^{-1}
S5	_/_	_/_	7.67	7.75	_/_	11.66	15.97	2.14
S10	_/_	11.85	7.67	8.39	8.72	12.41	15.32	2.55
S15	_/_	11.66	7.11	9.90	8.67	12.30	15.33	4.45
S20	14.47	11.81	6.74	10.17	8.05	11.21	15.07	5.04
S25	13.69	12.40	7.03	10.36	7.80	11.46	14.90	5.54
S30	16.43	12.75	8.23	13.03	8.24	_/_	14.62	6.13
S35	20.65	12.46	9.37	19.80	8.43	_/_	14.47	6.85

Reported values were means of three replicates (standard deviation).
Cooling and heating at 2°C min^{-1}; _/_: not exist.

3.3. Solid fat content of the different melting fractions

The SFC decreased with increasing temperature, as for seven fractions obtained after dry fractionation (Fig. 1). A lower SFC was observed for S5, with high concentration of short chain fatty acids and low concentration of long chain fatty acids (Table 1), in all temperatures evaluated. AMFs started to melt with the increasing temperature until it became liquid at a temperature above 30°C. S5 has 50 % of SFC at 5± 0.5°C and was totally melted (0 % SFC) at 30 ± 1.0°C, while S35 has 50 % of SFC at 15± 2°C and was totally melted (0 % SFC) at 35 ± 1.0°C. The differences between the average SFC values of these seven fractions were due to the differences in fatty acid composition between samples, which affected their melting points and enthalpies. Furthermore, the different melting fractions exhibited clearly different behavior in relation to the crystallization isotherm (Fig.2). At S15~S35, the slope of the second step is steeper and, hence, the α-β′ polymorphic transition seems faster than at lower

melting fractions (S5, S10). Furthermore, from S5 to S35, SFC curve was higher, this probably indicates the melting heat required to melt the crystals, created during each applied isothermal period, is higher, and differences in TAG-composition of the fat crystals or a combination thereof[16]. No significant difference in the final melting heat can be detected.
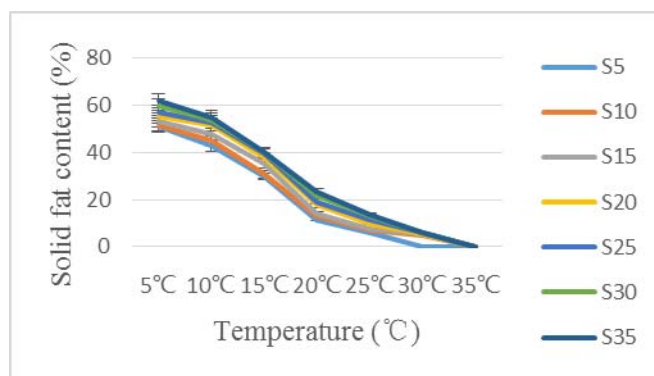

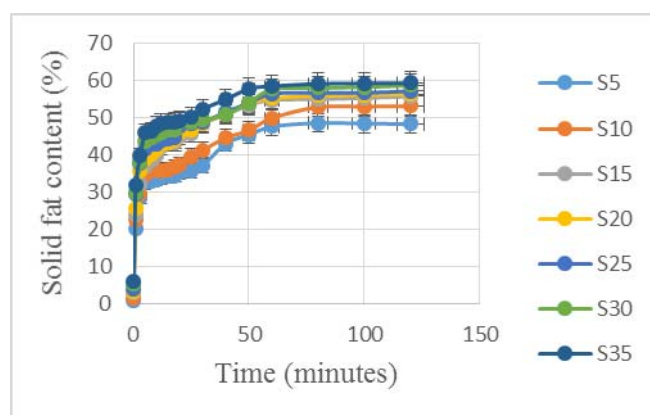**Fig. 1.** Solid fat content in different melting fractions (S35, S30, S25, S20, S15, S10, S5)

Fig. 2. Isothermal crystallization (4 °C) of different melting fractions (S35, S30, S25, S20, S15, S10, S5).

4. Conclusion

In this paper, effects of AMF on physicochemical composition, crystallization and thermal properties of the different melting fractions were studied. The results revealed that Enthalpies of melting and crystallization and the SFC profiles of different melting fractions in the present study are related to their fatty acid contents. This study also demonstrates the importance of formulating TAG blends in which their crystallization and melting behavior behave differently depending on chemical nature and amount of individual component to be incorporated. This information is necessary for certain applications, such as optimizing the amount of S5-S35 required to maintain oxidative stability of AMF and obtaining desirable texture and plasticity in AMF fortified dairy products. The type of polymorphs cannot be determined by DSC investigation. Study on the polymorphs of S5-S35 using X-ray diffraction technique will be covered in continuing research.

References

[1]   Lopez C, Bourgaux C, Lesieur P, et al. Milk fat and primary fractions obtained by dry fractionation 1. Chemical composition and crystallisation properties [J]. Chem Phys Lipids, 2006, 144(1): 17-33.

[2]   Kaylegian K E, Hartel R W, Lindsay R C. Applications of modified milk fat in food products [J]. Journal of Dairy Science, 1993, 76((6)): 1782-1796.

[3]   Pajin B, Sakač M, Simović D Š, et al. Influence of low‑melting milk fat fraction on crystallization and physical properties of chocolate [J]. British Food Journal, 2012.

[4]   Lopez C, Lesieur P, Bourgaux C, et al. Thermal and Structural Behavior of Milk Fat [J]. Journal of Colloid and Interface Science, 2001, 240(1): 150-161.

[5]   Ergun R, Hartel R W, Spicer P T. Kinetic effects on interfacial partitioning of fat crystals [J]. Food Structure, 2015, 5: 1-9.

[6]   Tolstorebrov I, Eikevik T M, Bantle M. A DSC determination of phase transitions and liquid fraction in fish oils and mixtures of triacylglycerides [J]. Food Research International, 2014, 58: 132-140.

[7]   Fredrick E, Van De Walle D, Walstra P, et al. Isothermal crystallization behaviour of milk fat in bulk and emulsified state [J]. International Dairy Journal, 2011, 21(9): 685-695.

[8]   Badan Ribeiro A P, Grimaldi R, Gioielli L A, et al. Thermal Behavior, Microstructure, Polymorphism, and Crystallization Properties of Zero Trans Fats from Soybean Oil and Fully Hydrogenated Soybean Oil [J]. Food Biophysics, 2009, 4(2): 106-118.

[9]   Truong T, Janin S, Li B-Z, et al. Effect of Crystalline Structure on Oxidation of Fish Oil in Stearin:Fish Oil Mixtures [J]. Food and Bioprocess Technology, 2016, 9(5): 792-800.

[10]   Knoester M, Vandente.M, Debruijn.P. The solid-liquid equilibrium of binary mixtures of triglycerides with palmitic and stearic chains [J]. Chemistry and Physics of Lipids, 1972, 9(4): 309-&.

[11]   Maki K C, Reeves M S, Farmer M, et al. Krill oil supplementation increases plasma concentrations of eicosapentaenoic and docosahexaenoic acids in overweight and obese men and women [J]. Nutr Res, 2009, 29(9): 609-15.

[12]   Kolanowski W, Ziolkowski M, Weißbrodt J, et al. Microencapsulation of fish oil by spray drying--impact on oxidative stability. Part 1 [J]. European Food Research and Technology, 2005, 222(3-4): 336-342.

[13]   Li B Z, Truong T, Bhandari B. Crystallization and melting properties of mixtures of milk fat stearin and omega-3 rich oils [J]. Food Chem, 2017, 218: 199-206.

[14]   Shen Z, Birkett A, Augustin M A, et al. Melting Behavior of Blends of Milk Fat with Hydrogenated Coconut and Cottonseed Oils [J]. Journal of the American Oil Chemists' Society, 2001, 78((4)): 387-394.

[15]   Tengku-Rozaina T M, Birch E J. Effects of fractionation on melting and crystallisation profiles of hoki oil measured by DSC [J]. Journal of Thermal Analysis and Calorimetry, 2014, 120(1): 395-402.

[16]   Fredrick E, Moens K, Heyman B, et al. Monoacylglycerols in dairy recombined cream: I. The effect on milk fat crystallization [J]. Food Research International, 2013, 51(2): 892-898.