Analyses of milk fat crystallization and milk fat fractions

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ABSTRACT
The aim of this study was to determine the possible extent of changes in the composition and physicochemical properties of unmodified milk fat (UMF) and to evaluate the influence of such modifications on the crystallization of solid fractions (SF) and liquid fractions (LF) obtained from UMF by dry fractionation at a temperature of 30°C, 25°C, and 20°C. The evaluation was based on the results of gas chromatography, differential scanning calorimetry (DSC), firmness/hardness values measured with a texture analyzer, and optical microscopy analysis. The results of the above analyses, in particular the evaluation of fatty acid (FA) composition, revealed that dry fractionation produces fractions with different composition and different ratios of saturated fatty acids (SFAs) to unsaturated fatty acids (UFAs). These variations also induced differences in the physicochemical properties of the analyzed samples, mostly changes in melting and solidification curves, as well as differences in the microstructure of milk fat (MF) analyzed under a microscope at a temperature of 10°C. The present findings indicate that FA composition is clearly correlated with firmness/hardness and the microscopically determined crystallization of MF and its fractions.

ARTICLE HISTORY
Received 21 September 2020
Revised 11 January 2021
Accepted 14 January 2021

KEYWORDS
Milk fat; fractionation; fractions; fatty acids; crystallization; firmness

Introduction

Consumer acceptance of butter is significantly influenced by its flavor and aroma as well as rheological properties. These properties and the extent to which they can be modified affect the spread-ability of butter and, therefore, have important practical implications. The rheological properties of butter are largely determined by the composition of triacylglycerols and fatty acids (FAs) in the fat phase, as well as the crystallization of milk fat (MF) which is controlled during the production process.

Milk fat is an edible fat with the most complex FA composition. More than 400 FAs have been identified in MF. However, only 16 FAs are present in large quantities, and they determine the physical properties of MF, including its melting and solidification temperature, content of the solid phase, and the firmness/hardness and spread-ability of the produced butter.

Milk fat is abundant in higher saturated fatty acids (SFAs) which account for 50–60% of total FAs. The proportions of short-chain (C4-C10) and medium-chain (C12-C14) FAs are also relatively high in MF. The content of monoenoic and polyenoic FAs in MF ranges from 26% to 36% and from 2% to 4%, respectively. The composition of FAs and triacylglycerol groups in MF varies considerably on a seasonal basis. Fatty acid composition is influenced by the animals’ diet, lactation period, individual traits, breed, health status, and weather conditions. The diet of dairy cows plays the most important role. However, animal diets are not modified to obtain fat with the most desirable qualitative traits for the production of butter. Seasonal fluctuations in the chemical composition of milk affect the physical properties of MF and, consequently, the produced butter. Due to differences in summer and winter feeding, butter produced in summer tends to be excessively soft, whereas butter produced in...
winter tends to be excessively hard. Therefore, the rheological properties of butter have to be standardized throughout the year. A sound knowledge of the physical and chemical properties of MF is essential in dairy practice.

The physical properties and, indirectly, the rheological properties of butter are largely determined by the structure of triacylglycerols, i.e. the distribution of FAs in positions sn-1, sn-2, and sn-3.[4,9,12] The distribution of FAs influences the melting temperature of low-melting (LMF), middle-melting (MMF), and high-melting (HMF) fractions.[4,8] The melting point of each triacylglycerol containing long-chain FAs is a product of at least three separate melting points of its main polymorphic forms $\alpha$, $\beta$ and $\beta'$.[3,4,13] These polymorphs differ mainly in the structure of the crystal network and resistance to changes in temperature. The hexagonal $\alpha$ form is least stable, and its crystallization during rapid cooling is reversible. The hexagonal form can be irreversibly transformed into a more stable orthorhombic $\beta'$ form and the most stable triclinic $\beta$ form. A highly unstable $\gamma$ form was also identified in microphotographic analyses.[13–15]

The content of different polymorphic forms is determined by the MF cooling method. Differential scanning calorimetry (DSC) analyses have demonstrated that structural changes in MF crystals can occur even at temperatures close to −40°C and +40°C.[3,14,15] At intermediate temperatures, MF is a mixture of solid fractions (SF) and liquid fractions (LF). The rheological properties of butter are determined mainly by the proportions of SF and LF in the fat phase.[13,16]

In dairy practice, the formation of MF crystals can be optimized during the physical maturation of cream to modify the rheological properties of butter and to compensate for seasonal variations in the chemical composition of MF. This process requires a thorough knowledge of melting and solidification temperatures of the main MF fractions.[5,12,13] Cream has to be matured to achieve the desired crystallization of MF (usually 30–50%) and promote adequate crystallization behavior in MF globules.[11,12]

The formation and growth of fat crystals are a highly complex non-linear process due to differences in the solidification temperature of various triacylglycerols and changes in crystal concentrations in the liquid phase over time. In MF globules, the space in which fat is crystallized is also limited by the presence of milk fat globule membranes.[4,6,14]

The composition and properties of MF can be modified to produce butter with the desired rheological attributes. The quantity and proportions of FAs can be changed through diet, for example by enriching animal feed with unsaturated fatty acids (UFAs), or by genetic engineering. The main aim of biological modification is to increase the content of UFAs, but these changes can also influence the size of MF globules and, consequently, the physicochemical properties of MF.[17–19] The physical properties of fats (and, less often, MF) are usually modified by hydrogenation, which involves a series of catalytic reactions to eliminate double bonds and produce harder fats, as well as re-esterification, an increasingly popular process that changes the location of FAs or introduces new FAs to triacylglycerol molecules.[20,21]

Fractionation is one of the most cost-effective methods of modifying the physical properties of MF.[7,22] During this process, fat is separated into fractions based on differences in melting temperature, solidification temperature, and volatility of triacylglycerols, as well as differences in the solubility of fat components.[4,23] Dry fractionation, solvent fractionation, and molecular distillation based on differences in molecular mass, melting temperature, volatility, and intermolecular interactions between triacylglycerols are interesting techniques that can be applied in fat production.[18,22] Supercritical CO$_2$ extraction is also a viable method for obtaining short-, medium- and long-chain triacylglycerol fractions of high purity.[17]

Dry fractionation without solvents is preferred as a more neutral method. The target crystallization temperature and cooling rates are controlled during the process. Fractions with different composition and properties can be extracted due to changes in temperature and a wide range of melting and solidification temperatures.[7,18,24,25] The obtained fractions are used in the food processing industry. Solid fractions are used mainly as additives in the production of cakes and pastes. They are also applied as hardening agents in the production of ghee and recombinant butter in tropical regions. Liquid
fractions enhance the spreadability of butter. Liquid fractions are also abundant in aromatic compounds, pigments, cholesterol, and vitamin A, and they are regarded as functional food ingredients ( nutraceuticals ) in baking, confectionary, chocolate and dairy industries. High-melting MF fractions prevent chocolate fat blooms. Low-melting MF fractions are applied to improve the texture of ice cream and processed cheese.

Solvent fractionation is an alternative fat extraction method. In this process, fat is dissolved in a solvent (such as acetone, isopropyl alcohol, hexane, pentane, or ethanol) before crystallization. The fractions obtained by dry fractionation and solvent fractionation differ in chemical composition. Their properties are determined by the applied solvent, and polar solvents are preferred. The main advantage of solvent fractionation over dry fractionation is that it significantly reduces processing time and facilitates the separation of crystals from LF. The main disadvantage of solvent fractionation is that lipids have to be refined to remove the solvent, which leads to the loss of their characteristic flavor and aroma. Solvent fractionation is also an expensive process, and it is cost-effective only in selected types of production, such as cocoa butter alternatives.

Unlike biological modification methods, physical modification of MF does not support direct standardization of the rheological properties of butter. Only the rheological behavior of the isolated MF can be modified. The extracted fractions have to be re-incorporated into the modified product. In industrial practice, this is usually achieved by recombination.

In view of the above, the aim of this study was to determine the possible changes in the composition and physicochemical properties of unmodified MF (UMF), and to evaluate the influence of such modifications on the crystallization of SF and LF obtained from UMF by dry fractionation.

Materials and methods

The possible modifications in the properties of MF were determined by analyzing changes in the composition of FAs and selected physicochemical properties of LF and SF. Milk fat fractions were derived from three batches of anhydrous MF with minimum 98% fat content, produced by a dairy plant in central-eastern Poland. The fractions were extracted by dry fractionation. The separation process was conducted in several steps at a temperature of 30°C/72h, 25°C/72 h, and 20°C/72 h (Figure 1). Before fractionation, anhydrous MF was completely liquefied at a temperature of 60°C in the BINDER KB 115 incubator with forced air circulation (until fully clarified). Milk fat was fractionated in the BINDER KB 115 incubator with forced air circulation. Solid fractions were separated from LF with the use of filter paper (Whatman, average flow rate) at each temperature (30°C, 25°C, 20°C).

Figure 1. Dry fractionation of milk fat without solvent at temperatures of 30, 25 and 20°C. TW – unmodified milk fat, FS – solid fraction, FP – liquid fraction.
Analytical methods

Analysis of fatty acid composition
The composition of FAs in MF samples was determined according to standard IDF 182 (1999). Milk fat was methylated in accordance with the IDF reference method: the sample was dissolved in hexane, KOH solution in ethanol was added, and the sample was shaken for 1 minute. Crystalline NaHSO$_4$ was added after 5 minutes. The sample was stirred, centrifuged at 1000 rpm for 3 minutes, and the transparent upper layer was separated in a gas chromatograph (7890A, Agilent Technologies, Palo Alto, California, USA) with a flame ionization detector. Chromatographic separation was performed under the following conditions: CP-Sil 88 capillary column (100 m, 0.25 mm, 0.20 µm) (Agilent Technologies, Palo Alto, California, USA); thermal gradient from 60°C (1 min) to 180°C at 5°C/min; injector temperature – 225°C; detector temperature – 250°C; carrier gas – helium; gas flow rate – 0.8 ml/min; sample to sorbent ratio: 1:50; sample volume – 1 µl.

The results were expressed as the percentage content of individual FAs in total FAs (% by weight).

Crystallization curves based on differential scanning calorimetry data
The energy flows associated with melting and solidification of MF, LF, and SF were evaluated based on the endothermic and exothermic effects of melting and solidification in the Universal Analysis 2000 program (TA Instruments, New Castle, DE, USA). The samples were placed in tightly closed aluminum pans (10 mg) and were analyzed in the Q10 differential scanning calorimeter (TA Instruments, New Castle, DE, USA). An empty aluminum pan was the reference. The samples were analyzed under the following conditions: temperature of −40°C to +40°C; gas atmosphere – nitrogen 5.0; rate of temperature change ∆t = 5°C·min$^{-1}$. 

Figure 2. Average content of fatty acids in milk fat samples (TW), liquid (FP) and solid (FS) fractions obtained at 30, 25 and 20°C (n=3).
**Instrumental Texture Analysis**

Fat firmness/hardness was determined with the TAXT Plus texture analyzer coupled with Exponent Connect software (Stable Micro System, UK). The measurement was performed with a P/5 cylinder probe that measures penetration-firmness to a depth of 12 mm. Fat samples (in aluminum containers) were tempered in a water bath inside the BINDER KB 115 incubator at 10°C for 24 h.

**Microscopic analysis**

Crystallization was analyzed in samples of UMF and in the obtained LF and SF. Fat samples were analyzed under the AMPLIVAL (Zeiss Jena) optical microscope equipped with a polarization set and the CANON EOS 550D digital camera with a resolution of 18 mpx. The microscope was coupled with the SEMIC cooling and heating module. Samples of 50 cm$^2$ were liquefied at a temperature of 60°C and tempered in the BINDER KB 115 incubator at 10°C for 4 days. Tempered samples were transferred to slides with a specimen pin and held in place with a coverslip. The microscopic analysis was performed at a constant temperature of 10°C. A 50 g weight was applied to cover slips for 1 minute. Light was transmitted through the specimen, and bright-field images were acquired.

**Results and discussion**

**Fatty acid composition**

In an analysis of the FA profile of UMF, the average proportions of SFAs and UFAs were determined at 69.19% and 30.81%, respectively (Figure 2). Four main FA groups were identified: volatile FAs, higher SFAs, monoenoic FAs, and polyenoic FAs. Their proportions in total FAs were determined at 8.17%, 61.02%, 28.29%, and 2.52%, respectively. Palmitic acid (C16:0) was the most abundant FA which accounted for 32.02% of total FAs on average.

Blasko et al.\[26\] reported a high correlation between the FA profiles of milk and butter. Winter milk is more abundant in SFAs than summer milk, and in analyses conducted at the same temperature, butter produced during the winter season is characterized by higher hardness and lower spreadability than butter made in the summer.\[10, 12\] Similar results were reported by Felkner-Poźniakowska et al.\[10\] in whose study, winter milk contained 71.90% SFAs and 30.69% UFAs.

Fatty acid composition exerts a considerable influence on the quality and consistency of butter, which are generally referred to as firmness/hardness and spreadability. A higher content of UFAs increases the nutritional value of butter and contributes to its softness. A low content of SFAs, including long-chain FAs (C11:0 – C20:0), decreases the melting temperature of MF in comparison with fat that is abundant in long-chain SFAs.\[27\] In all three samples of UMF (UMF1, UMF2, UMF3), butanoic acid (C4:0) was the predominant volatile FA; palmitic acid (C16:0) was the most prevalent higher SFA; oleic acid (C18:1 cis) was the most abundant monoenoic FA, and linoleic acid (C18:2) was the predominant (1.3–1.4%) polyenoic FA.

Considerable differences in composition and physicochemical properties were observed between LF and SF, and between both fractions and UMF. The total content of SFAs was higher in SF than in LF (at all temperatures – 30°C, 25°C, and 20°C). The SFA:UFA ratios in both fractions, at all analyzed temperatures, are presented in Figure 3.

Solid fractions SF30 and SF25 were characterized by the highest total content of SFAs which was determined at 73.14% and 71.18% on average, respectively. The highest content of UFAs was noted in liquid fractions LF25 and LF20 at 33.17% and 33.28% on average, respectively. These results indicate that MF fractionation increased the content of SFAs (mainly higher SFAs) in SF, and the content of UFAs (monoenoic and polyenoic) in LF. Liquid fractions also contained higher concentrations of volatile FAs than UMF.

Fractionation is an effective modification technique that improves the functional properties and the nutritional value of MF. The content of triacylglycerol fractions can be modified to produce butter with the desirable texture. Butter, which is spreadable at refrigerator temperature, can be obtained by...
increasing the content of LMF and decreasing the content of HMF. However, the resulting product will have semi-liquid consistency at room temperature.  

Recombined butter with optimal proportions of different fractions has the most desirable spread-ability and firmness/hardness. The present results indicate that a higher content of solid fractions (SF30 and SF25) increases the firmness/hardness of butter at room temperature, whereas a higher content of liquid fractions (LF25 and LF20) increases spreadability and softness.

**Crystallization and melting curves (DSC analysis)**

The results of DSC analysis can be used to describe the kinetics and mechanism of crystallization (melting/crystallization temperature, specific heat capacity, and, indirectly, the polymorphism of fat crystals). During the DSC analysis, samples of UMF and MF fractions were first melted within a temperature range of −40°C to +40°C, and then they were crystallized within a temperature range of +40°C to −40°C at a rate of 5°C min⁻¹. Examples of melting and crystallization thermograms for UMF and MF fractions are presented in Figure 4. One to three exothermic peaks (during crystallization) and 1–2 endothermic peaks (during melting) were identified in the analyzed fractions. Curve shape and peak height differed in samples with varied FA composition, which is consistent with the findings of Razavi et al.

Milk fat crystallizes and melts in several stages, which correspond to the peaks in the thermal analysis. Thermal peaks are highly correlated with the rates of heating/cooling. Individual peaks are analyzed by examining three partially overlapping peaks that correspond to LMF, MMF, and HMF. However, such analyses do not support the visualization of all thermal transitions during DSC.

According to Razavi et al., butter contains two triacylglycerol groups. The first group is abundant in polyenoic FAs, and the second group is characterized by high concentrations of SFAs. In melting curves, the corresponding peaks are located between the temperatures of 11.9°C and 13.1°C, and 30.2°C and 32.9°C. In the current study, MF was analyzed at a relatively high rate of changes in temperature (5°C/min) when peaks typically overlap. As a result, fewer individual peaks were observed. According to Campos et al., the number of individual peaks is higher when temperature changes at a slower rate. The UMF samples contained two main groups of triacylglycerols, which crystallized at 6.4°C and 12.8°C. This implies that UMF should be most abundant in SF at 6.4°C.
a temperature of <20°C and that it should be characterized by high firmness/hardness. The melting curve analysis revealed a single peak with a maximum value at 14.4°C, which was characteristic of MMF. Similar observations were made by Smiddy et al.\textsuperscript{[30]} 

\textbf{Firmness (hardness)}

The firmness/hardness of samples provides information about the rheological properties of MF and, indirectly, its spreadability. Other authors reported strong correlations between firmness/hardness, spreadability and the content of SFAs in fat.\textsuperscript{[1,14]} Saturated fatty acids are responsible for the rigidity and plasticity of fat, and they contribute to the formation of a three-dimensional crystal network, which retains LF. However, the rheological properties of fat (including firmness/hardness) are also influenced by other factors, such as the number of crystals in the network, crystal size, morphology, and polymorphism. Numerous studies have demonstrated that crystallization of melted fat (including
crystallization parameters such as cooling and storage conditions) significantly influences fat firmness/hardness.\cite{1,4,14}

In the texture analysis performed with the TAXT Plus texture analyzer, the average firmness/hardness of MF samples was determined at 13.8 N. As shown in Figure 5, SF was characterized by higher firmness/hardness, which can be attributed to differences in the content of SFAs (Figure 3). The SF obtained at a temperature of 30°C was characterized by the highest firmness/hardness (25.4 N on average), and it had the highest content of SFAs. The LF obtained at a temperature of 20°C was characterized by the lowest firmness/hardness (11.1 N on average), and it had the lowest content of SFAs in the group of the analyzed LF.

Liquid fractions were more abundant in monoenoic and volatile FAs than SF. The highest concentrations of these FAs were determined in the LF obtained at a temperature of 20°C (31.4% and 9.2%, respectively) (Figure 3). This LF was also characterized by the lowest firmness/hardness (Figure 5). Firmness/hardness tended to be lower in liquid fractions with a higher content of monoenoic and volatile FAs.

The results of the texture analysis revealed the presence of correlations between crystallization determinants (including FA composition) and firmness/hardness parameters. According to Campos et al.,\cite{14} these correlations can be partially attributed to differences in the number of crystallization centers in UMF and in SF and LF acquired at different temperatures (30°C, 25°C, 20°C). The presence of considerable differences between MF and the isolated fractions was confirmed in the microscopic analysis.

**Microscopic analysis**

Microscopic images of UMF as well as SF and LF extracted at different temperatures (30°C, 25°C, 20°C) were acquired at 10°C and presented in Figure 6. The microscopic analysis of UMF revealed the presence of large crystal formations with liquid fat in the spaces between structures. All crystal formations were intersected by visible lines with a crisscross pattern.

Crystal formations were generally classified into two groups. The first group comprised granular microstructures with a large number of small crystals, whereas the second group contained granular microstructures with large crystals that were less densely packed in the field of view. Liquid fractions were characterized by a large number of small crystals. The fractions obtained at higher temperatures contained smaller crystals (the crystals in FP30 had a smaller diameter, image B) than those obtained

![Figure 5. Average firmness (hardness) of milk fat samples (TW), liquid (FP) and solid (FS) fractions obtained at 30, 25 and 20°C (n=3).](image-url)
at lower temperatures (FP25 and FP20, images C and D, respectively). In turn, SF contained fewer crystals with much larger diameters (images E, F, and G).

According to Campos et al., granular structures composed of a large number of small crystals are generally formed when MF is rapidly cooled. Nucleation proceeds rapidly when the induction time for crystallization is short. Rapid crystallization is accompanied by an increase in viscosity, which inhibits crystal growth. When crystallization proceeds at a slow rate, large crystals and crystal aggregates are formed, and the space in the crystal network is filled with liquid fat or very small seed crystals. The results of the present study indicate that crystal distribution plays an important role during the crystallization process. Crystals are more uniformly distributed when crystallization proceeds at a slower rate, which influences penetration (hardness). It should be noted, however, that samples of SF and LF were cooled under identical conditions in this study. According to Campos et al.,
crystals with a uniform microstructure are more resistant than non-homogeneous samples (containing liquid fat in the spaces between crystal formations). In the current study, fractions with more uniformly distributed crystals were characterized by higher firmness/hardness than fractions with a higher number of spaces containing liquid fat at the tested temperatures (Table 1).

**Conclusion**

The following conclusions can be drawn from the presented analyses of anhydrous MF and the extracted SF and LF: (i) SF and LF with different properties and different processing suitability can be easily extracted from UMF by dry fractionation, (ii) Solid fractions are characterized by a higher content of SFAs and higher firmness/hardness than LF and UMF, (iii) Differences in the SFA:UFA ratio influence the physicochemical properties of MF fractions. Solid fractions with a higher content of SFAs (in particular SF30) are characterized by higher firmness/hardness and have a granular microstructure with larger and less densely packed crystals. Liquid fractions (in particular LF25 and LF20) are characterized by lower firmness/hardness and have a granular microstructure with numerous

| Crystallization image | Crystallization image | Crystallization image | Crystallization image |
|-----------------------|-----------------------|-----------------------|-----------------------|
| TW                    | FP30                  | FS30                  | FP20                  |
| TW                    | FP30                  | FS30                  | FP20                  |
| TW                    | FP30                  | FS30                  | FP20                  |
| TW                    | FP30                  | FS30                  | FP20                  |

Table 1. Crystallization images, firmness values, and SFA:UFA ratio of samples (milk fat and fractions FP30, FP25, FP20 and FS30, FS25, FS20).
small crystals, and (iv) The present study revealed clear correlations between FA composition, firmness/hardness, the SFA:UFA ratio and the microscopically determined structure of crystal networks in MF and MF fractions.

Acknowledgment

Project financially supported by The National Centre for Research and Development, Project No. WPC1/DairyFunInn/2019, amount of funding 1.950.000,00 PLN.

Funding

Project financially supported by The National Centre for Research and Development, Project No. WPC1/DairyFunInn/2019, amount of funding 1.950.000,00 PLN.

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List of Abbreviations

MF – milk fat
UMF – unmodified milk fat
SF – solid fraction
LF – liquid fraction
LMF - low-melting fraction
MMF - middle-melting fraction
HMF - high-melting fraction
FA – fatty acid
SFA – saturated fatty acid
UFA – unsaturated fatty acid

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