Effects of variation in fatty acids and triglyceride composition on melting behavior in milk fat

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Date submitted:11/09/2017 *Date accepted: 18/01/2018* *Volume/Page(s): 71/4-9*

Abstract

The aim of this study was to determine the effects of fatty acids and triglycerides composition on melting behavior of milk fat. The melting profiles, fatty acids composition and triglycerides composition of milk fat from 27 cows fed palm oil, rapeseed oil and a low fat diet were analyzed. Correlations between individual fatty acids and triglycerides, and the melting off-set temperatures of milk fat fractions were obtained using multivariate data analysis. Low fat feeding increased the proportion of short and medium chain fatty acids and decreased the proportion of long chain fatty acids. The opposite effect was observed in milk fat of cows from lipid supplemented diets. Palmitic and myristic acids correlated positively to the melting off-set temperatures of high melting fraction of milk fat while oleic and linoleic acid had strong negative correlations. Furthermore, high molecular weight triglycerides contributed positively to the melting off-set temperatures of high melting fraction of milk fat. This is the first study to demonstrate that the melting off-set temperatures of high melting fraction of milk fat can be affected, to some extent, by dietary manipulations of cows. However, melting off-set temperatures of milk fat were still under physiological temperatures of cows indicating that the biological mechanism secure full milk fluidity under most dietary manipulations. Knowledge from this study could help to characterize melting fractions of milk fat resulting from different feeding regimes. This could aid in milk fat fractionation for use in specialty ingredients or modification of functionality of milk fat (e.g. softness or spreadability) in fat-based products.

Introduction

Lipids constitute 3-5% of bovine milk. Milk fat is the main determining factor for the textural and flavour characteristics of dairy fat based products. Fat in milk is present in the form of globules containing triglycerides (TAG) cores. TAG form approximately 98% of milk fat and are esters of glycerol and fatty acids (FA). TAG are numbered from 26 to 54 based on the total number of carbons of fatty acyl units on the glycerol backbone. Furthermore, milk fat is classified into low melting fraction (melting temperature <10 °C), medium melting fraction (melting temperature between 10 °C and 25 °C) and high melting fraction (melting temperature > 25 °C). The melting and crystallization mechanisms as well as the rheological properties of milk fat are affected by the structure of TAG [1]. In addition, crystallization and melting behavior are dependent on the degree of unsaturation of milk fatty acids and their carbon numbers [2]. De novo synthesis is the main source of short and medium chain fatty acids, including part of C16:0, in milk. Long chain fatty acids originate from the diet [1]. The fatty acid composition of milk fat is highly variable. One of the main factors that affect the composition of milk fatty acids is the diet of cows [3,4,5]. Changes in the fatty acids composition of milk fat are expected to affect not only the technological properties of milk fat (e.g. such as texture of milk-fat based products) but also nutritional and organoleptic properties of products.

Studies on changes in fatty acids composition of milk fat, and its impact on the technological properties of milk fat are quite elaborate [6,7,8]. It is known historically that summer butters are softer than winter butters. Grazing of cows during summer enriches milk fat with unsaturated fatty acids, which have low melting temperatures and that account for the softer nature of summer butters [8]. Additionally, an increased content of unsaturated fatty acids in milk fat has been shown to influence the cold spreadability of butter [6]. Moreover, feeding cows with extruded linseed (60% of concentrate) led to a decrease in saturated fatty acids from 71% to 61%. This was accompanied by decreases in melting points of 3.8°C, 1.6°C and 1.7°C for low-melting, medium-melting and high-melting fractions of milk fat respectively [7]. Significant correlations between individual FA and melting temperatures of medium melting fraction but not for the high melting fraction of milk fat have been reported [9]. Similarly, Larsen et al. [8] obtained significant correlations between FA and melting temperatures of low melting fraction of milk fat but not the medium melting or high melting fraction of milk fat. Other factors such as breed, lactation stage, lactation number, health status, seasons, and fasting (ketosis) may also to some extent affect the fat composition of milk [8]. The aim of this study was to determine the effects of FA composition, as affected by feeding of cows, on melting behavior of milk fat. This is because some authors have suggested that it might be difficult to affect melting temperatures of the higher melting fractions of milk fat due to a requirement to keep milk fat fluid at physiological temperatures of cows. In fact, only one study has shown that it could be possible to induce changes in the said fractions of milk fat, though such changes could be minimal [7]. Therefore, the influence of dietary changes on the melting temperatures of milk fat, particularly high melting fraction (HMF) and medium melting fraction (MMF), still remains to be fully comprehended. Furthermore, as milk fat fraction can be separated and used as ingredients in for

Milk Science International (71) 2018 P. 4-9
ISSN 2567-9538

example confectionery products, it is important to know how the individual fractions can be manipulated through feeding.

Knowledge from this kind of study could help to characterize melting fractions of milk fat resulting from different feeding regimes, which could help in milk fat fractionation. Owing to the complexity of the FA and TAG compositions of milk fat, multivariate data analyses was used to extract correlations between FA, melting off-set temperatures and solid fat content of milk fat. This approach gave a better understanding of the impact of fatty acids composition on melting temperatures of milk fat.

Materials and methods

Description of milk samples:

Milk were sampled from cows in research herds from Denmark (low fat diet group), and Sweden (palm oil or rapeseed oil supplemented cows) twice daily in morning and evening. All cows were healthy and treated under the same conditions except for feeding as described next. High rapeseed or palm fat diet milk samples were from an experiment with Swedish Red cows in mid lactation. Silage was fed ad libitum and consumption amounted to 51% silage on dry matter (DM) basis, 3.9% crude fat in DM, and 49% concentrate 7.8% crude fat in DM. Concentrate composition was rape seed meal 27.7%, wheat 19.9%, oats 15.0%, wheat meal 10.3%, barley 7.0%, dried sugar beet pulp 6.0%, palm kernel expeller 4.0%, palm or rapeseed oil 4.0%, soy bean meal 2.0%, beet molasses 2.0% and minerals 2.1%. Eleven milk samples from individual cows fed the palm oil supplement, and nine milk samples from the cows fed the rapeseed oil were included in this study. Low fat diet milk samples were from an experiment with Danish Holsteins cows in early to mid-lactation. Rations were fed ad libitum and consisted of (on DM basis) 60% ryegrass silage (3.2% crude fat in DM) and 40% concentrate (1.9% crude fat in DM). Concentrate composition (as is basis) was barley 44.8%, soybean meal 27.7%, dried sugar beet pulp 24.3%, and minerals 3.3%. Seven milk samples from individual cows fed the low fat diet were included.

Isolation of fat:

Fat from milk samples was isolated and used for thermal analysis, fatty acids and triacylglycerols composition analyses, and solid fat content determination. The milk samples were centrifuged at 4°C for 30 min at a speed of 2000 g. The resulting cream layers were transferred into 1.5 ml eppendorf tubes and centrifuged at room temperature for 10 min at 3700 g. The eppendorf tubes with their contents were then left on a heating block for between five minutes to 10 min at 60°C after which another centrifugation was done at 40°C for 10 min at 3700 g. The fat samples were collected and stored at -18°C until further analysis.

Thermal Analysis of milk fat:

Non-isothermal crystallization curves for milk fat samples were obtained by using differential scanning calorimetry (DSC) (Q2000, TA Instruments, New Castle, DE). The DSC was calibrated with indium and then nitrogen gas was used to purge the flow system. Approximately, 10 mg to 15 mg of liquid fat was transferred into a hermetic aluminum pan while using an empty pan as a reference. The time-temperature program was as follows; liquid milk fat was first held at 65°C for 15 min to clear any crystal memory. Cooling at a rate of 10°C/min to -40°C, and holding isothermally at that temperature for five minutes was followed. Thereafter, the crystallized fat samples were melted by increasing temperature to 70°C at a heating rate of 20°C/min. Crystallization onset and melting off-set temperatures were determined using Universal Analysis software (TA Instruments). Temperatures of crystallization onsets and melting off-sets as well as melting fractions were subsequently identified [10].

Table 1: Mean ± SEM of fatty acid composition, crystallization onset temperatures, melting off-set temperatures and solid fat content for milk fat from cows fed palm oil and rapeseed oil or low fat diets

Parameter	Rapeseed oil diet	Palm oil diet	Low fat diet	*P*- value
Number of samples	**9**	**11**	**7**	
FA (% of total FA)				
C4 to C12	16.43± 0.37**a**	16.66± 0.50**a**	19.17± 0.28**b**	<0.05
C11 to C17	1.42 ± 0.09**a**	1.47 ± 0.04**a**	2.73± 0.03**b**	<0.05
C14:0	11.02±0.21**a**	11.45±0.22**a**	13.34±0.12**b**	<0.05
C14:1	1.00±0.06**a**	1.06±0.05**a**	1.24±0.02**b**	<0.05
C16:0	23.11±0.63**a**	24.46±0.51**a**	36.83±0.20**b**	<0.05
C16:1	1.03±0.05**a**	1.17±0.05**a**	2.21±0.05**b**	<0.05
C18:0	11.85±0.37**a**	11.12±0.35**a**	6.69±0.11**b**	<0.05
C18:1 trans-11	0.37±0.01**a**	0.33±0.01**a**	0.17±0.02**b**	<0.05
C18:1 cis-9	25.00±0.52**a**	23.66±0.57**a**	12.93±0.34**b**	<0.05
C18:2n-6	2.38±0.13**a**	2.44±0.19**a**	1.21±0.01**b**	<0.05
C18:3n-3	0.85±0.03**a**	0.83±0.03**a**	0.43±0.02**b**	<0.05
CLA cis-9, trans-11	0.89±0.05**a**	0.82±0.03**a**	0.53±0.05**b**	<0.05
Crystallization onset (°C)	12.96±0.62	14.47±0.80	14.2±0.21	NS
HMT (°C)	34.22± 0.97**a**	34.60± 0.66**a**	38.00± 0.16**b**	<0.05
MMT (°C)	17.58± 0.31**a**	18.17± 0.43**a**	19.73±0.19**b**	<0.05
SFC (22°C)	4.96±1.16**a**	6.46±1.03**a**	16.84±0.48**b**	<0.05
SFC (25°C)	1.18±0.82**a**	1.41±0.42**a**	4.81±0.99**b**	<0.05
SFC (26.5°C)	0.77±0.66**a**	0.81±0.32**a**	7.00±0.80**b**	<0.05

SEM = Standard error of the mean for measurements. Numbers which have the same alphabets, in a row, are not significantly different from each other at a 0.05 significance level.

Determination of FA composition of milk fat:

Fatty acid methyl esters were prepared from milk fat and analyzed according to the gas chromatography with flame ionization detection (GC-FID) method previously described [5] except that heptane was used instead of pentane.

Determination of TAG composition of milk fat:

Milk fat samples were prepared for triglycerides composition analysis in accordance to International Standards Organization (ISO) method 17678 [11]. Analysis of TAG composition was carried out as previously described in literature [8].

Determination of solid fat content of milk fat (direct method):

Liquid fat was transferred into nuclear magnetic resonance (NMR) tubes and held at 65°C for one hour to clear any crystal memory. The tubes with their contents were incubated in water baths set at specified crystallization temperatures (25°C and 22°C) for three hours, and 33°C, 30°C, 28°C and 26.5°C for 15 h. Thereafter, solid fat content was determined by use of a pulsed-NMR (Oxford Instruments, UK) with a daily calibration.

Statistical analysis:

Data from the thermal analysis of milk fat by use of DSC, FA compositions, TAG compositions and solid fat content of milk fat were analyzed

for significant difference between feeding methods by univariate analysis, at a significance level of 0.05 using R statistical program version 3.3.2 (R foundation for statistical computing, Austria). Multivariate data analysis was performed by principal component analysis (PCA) and partial least square (PLS) analysis in SIMCA P+ (Umetrics, Sweden). Correlations between FA and melting off-set temperatures, and between FA and TAG compositions were determined through PLS analysis.

Using PLS analysis, it was possible to correlate one or more responses (Y variables) to several predictors (X variables). Response variables in this work included crystallization onset temperatures, melting off-set temperatures of medium and high melting fractions of milk fat, solid fat content (at 22°C, 25°C and 26.5°C), and TAG. The predictor variables were FA and TAG compositions. All models from PCA and PLS were cross-validated. Variables were scaled to unit variance and centered during analysis.

Results and Discussion

Differences in FA and TAG profiles:

The main focus of this study is to report effects of large variations in fatty acid composition on melting behavior thus milk fat was obtained from different cow breeds and from different experimental cow feeding studies. Tables 1 and 2 show the FA and TAG compositions respectively of milk fat samples from different feeding regimes. Moreover, the PCA scores and loading plots provide pictorial views of differences in FA and TAG compositions of milk fat from different feeding methods (Figures 2 and 3). A significant difference in FA or TAG composition of milk fat is depicted by a clear separation between groups. Milk fat samples from the low fat group were clearly separated from milk fat samples in palm oil and rapeseed oil groups (Figure 1a). There was no clear separation between milk fat samples from palm oil feeding and rapeseed oil feeding. The loading plot of PCA of FA (Figure 1b) confirms significantly higher proportions of C18 based FA such as C18:0, C18:1 cis-9, C18:1 trans-11, C18:2n-6 and C18:3n-3 but significantly lower proportions of FA such as C14:0 and C16:0 in milk fat from palm oil and rapeseed oil feeding compared to low fat feeding. Regarding TAG composition, milk fat from the low fat diet was richer in TAG C32 to C38 and TAG C42 to C46 but contained lower proportions of TAG C50 to C54 than milk fat from rapeseed oil and palm oil diets (Figure 2a & b). Like the present results, previous studies have reported that dietary fat supplementation decreases yields of short and medium chain FA and increases proportions of long chain fatty acids (LCFA) [12,13]. Besides the observed influence of dietary fat supplementation on proportions of FA and TAG in milk fat, the total fat content of milk could be affected by feeding but this requires further investigation.

Table 2: Mean ± SEM of triglyceride composition (% relative distribution) of milk fat from cows fed low fat, palm oil or rapeseed oil diets

TAG carbon number	Rapeseed oil diet	Palm oil diet	Low fat diet	P-value
Number of samples	9	11	7	
C26	0.55±0.03	0.56±0.02	0.53±0.01	NS
C28	0.61±0.04	0.62±0.03	0.62±0.01	NS
C30	1.03±0.05	1.08±0.05	1.22±0.03	0.05
C32	2.12±0.07a	2.28±0.10a	2.87±0.05b	<0.05
C34	4.88±0.13a	5.20±0.16a	7.64±0.11b	<0.05
C36	8.74±0.22a	9.02±0.27a	13.17±0.22b	<0.05
C38	10.92±0.29a	10.74±0.31a	11.89±0.20b	<0.05
C40	9.29±0.24a	8.88±0.27ab	8.36±0.13b	<0.05
C42	5.66±0.18a	5.98±0.16a	7.99±0.23b	<0.05
C44	5.74±0.22a	6.42±0.11b	8.72±0.23c	<0.05
C46	7.60±0.20a	8.24±0.18b	9.94±0.19c	<0.05
C48	9.80±0.21a	10.30±0.29ab	10.85±0.23b	<0.05
C50	13.03±0.28a	13.03±0.35a	10.57±0.32b	<0.05
C52	12.28±0.34a	11.60±0.41a	5.09±0.26b	<0.05
C54	7.76±0.51a	6.09±0.28b	0.59±0.10c	<0.05

SEM = Standard error of the mean for measurements. Numbers which have the same alphabets, in a row, are not significantly different from each other at a 0.05 significance level.

Correlations between FA and TAG:

The TAG compositions obtained in this study reflected the FA compositions of milk fat from different feeding experiments. This observation was confirmed through PLS analysis which established significant correlations between FA and TAG (Figure 3).

TAG such as C32, C34, C36, C42, C44 and C48 were positively correlated to FA such as C14, C16, C4 to C12 and C11 to C17. In addition, these TAGs were positively related to C16:1 and C14:1. and negatively related to C18, C18:1 cis-9, C18:2 n6-cis, C18:3 n3, C18:1 trans-11 and conjugated linoleic acid (CLA cis-9, trans-11). TAG C40 and the higher

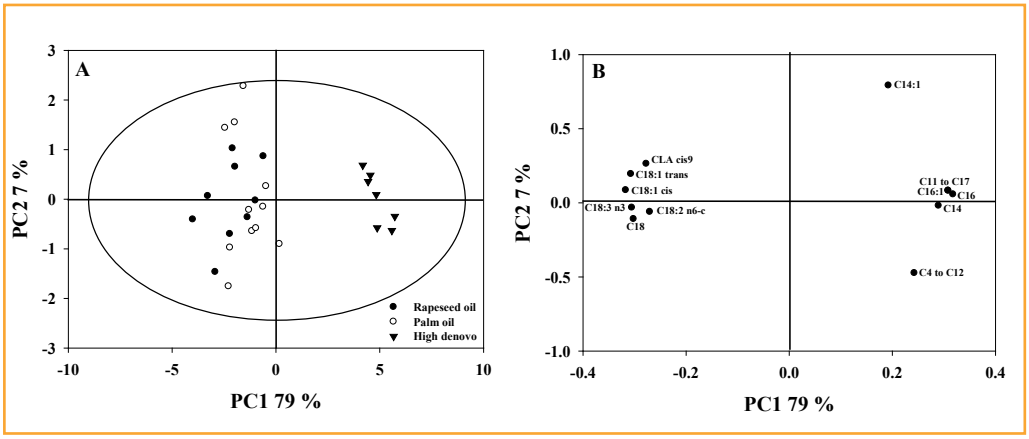

Figure 1: a) Principal component analysis (PCA) scores of Fatty acids (FA) and b) loading plot of PCA of FA composition of milk fat from cows fed rapeseed oil, palm oil or de novo (low fat) diets. Model Parameters: R2 = 0.86, Q2 = 0.72. The percentage value in PC1 is the explained variation in FA composition of milk fat from cows in different feeding groups. The percentage value in PC2 is the explained variation in FA in individual cows from the same group.

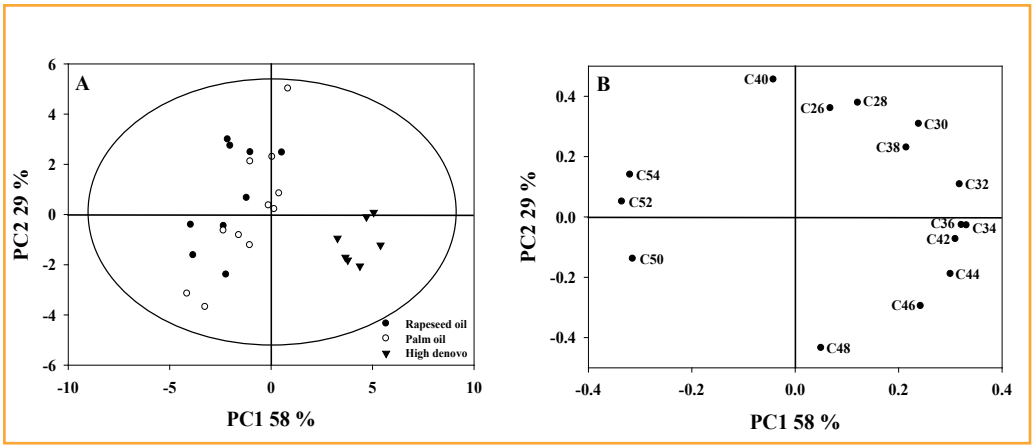

Figure 2: a) PCA scores and b) loading plot of TAG compositions of milk fat from cows fed rapeseed oil, palm oil or de novo (low fat) diets. Model parameters: R2 = 0.87, Q2 = 0.80

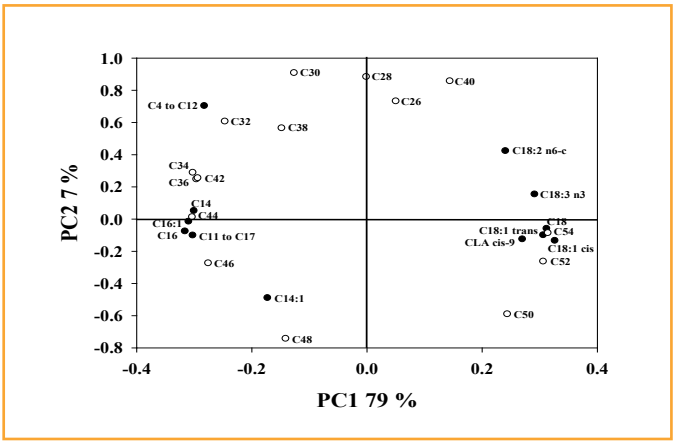

Figure 3: Partial least square (PLS) loadings for identification of correlations between FA (X) and TAG (Y). Model parameters: X2 = 0.86, Y2 = 0.78, Q2 = 0.71. c = cis. FA is closed circles and TAGS are open circles. CLA = conjugated linoleic acid. R2 = 0.78, Q2 = 0.71

molecular weight TAG (C50, C52 and C54) had the reverse correlation. These observations could imply the use of short and medium chain FA for the synthesis of low to medium molecular weight TAG, and long chain FA for the synthesis of high molecular weight TAG. Nonetheless, a detailed analysis of the molecular species of TAG from the different feeding regimes is still needed to affirm these assertions.

Differences in thermal properties of milk fat from feeding methods: Milk fat from cows fed the low fat diet had significantly higher melting temperatures than milk fat from cows fed rapeseed oil and palm oil diets (Table 1). The melting temperatures of milk fat from the high melting fraction of milk fat (HMT) from the low fat diet group were 3.4-3.8°C higher than the melting temperatures of milk fat of the same fraction from cows on the two other diets. However, the medium melting fraction of milk fat (MMT) was only 1.6-2.2°C higher when comparing milk fat from low fat diet group to the fat supplemented groups. Solid fat content was also significantly higher, at both 22, 25 and 26.5°C, for milk fat from low fat feeding compared to lipid supplementation. As expected, melting off-set temperatures and solid fat content were aligned. Thus, the present study demonstrates that a low fat diet, which led to milk fat composition being richer in short and medium chain FA (PCA plot in Figure 1a & b) actually increases the melting temperatures and solid fat content more than fat supplemented diets. We have earlier reported that feeding increasing level of palm fatty acid distillate fat to dairy cows did not increase C16 content in milk but reduce on the de novo fat synthesis [14]. Whereas, a mixture of C16 and C18 based fat supplements results in more long chain fatty acids and increase solid fat contents [15]. Feeding cows with linseed concentrate enrich milk fat with unsaturated fatty acids, and consequently decrease melting temperatures and solid fat content [7]. However, in the present study, the effect of rapeseed oil was modest.

At temperatures above 26.5°C, no solid fat content could be detected in almost all milk fat samples unless a very long incubation time (>15 h) was applied before measurement (data not shown). Importantly, the almost lack of crystallization and hence low solid fat content at temperatures close to physiological temperature (39°C) of cows could have biological relevance because it is a requirement to keep milk fat fluid

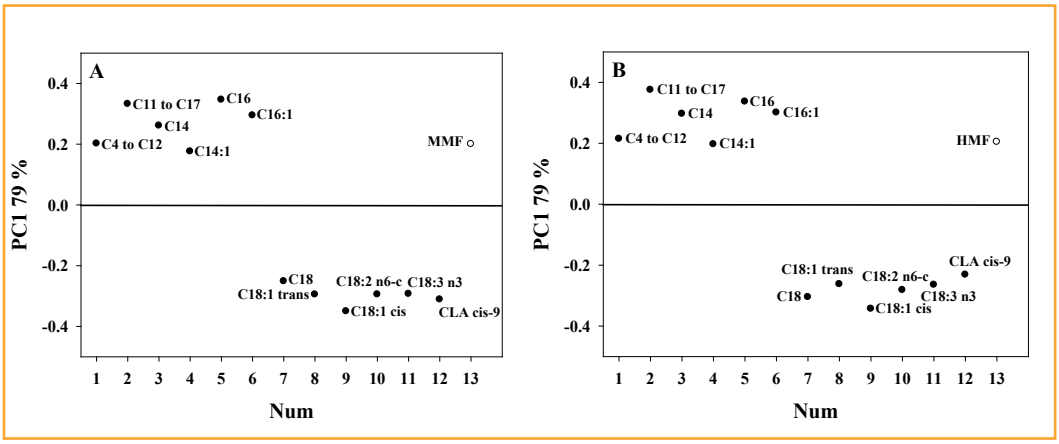

Figure 4: PLS loadings for identification of correlations between A) FA (X) and MMF (Y) and B) FA (X) and HMF. FA is closed circles; MMF and HMF are open circles. Model parameters: A) X2 = 0.83, Y2 = 0.55, Q2 = 0.51, R2=0.33 B) X2 = 0.83, Y2 = 0.52, Q2 = 0.49, R2=0.39. c = cis. CLA = conjugated linoleic acid. Num = number of data points.

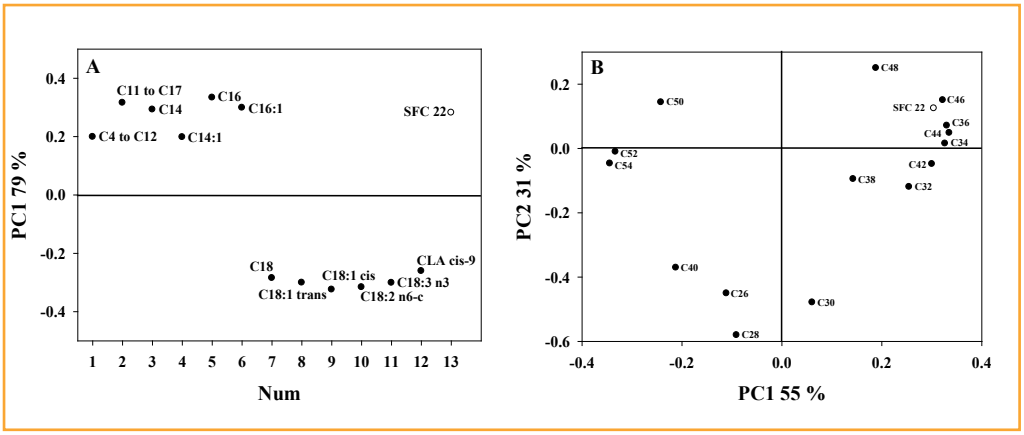

Figure 5. PLS loadings for identification of correlations between A) FA (open circles) and solid fat content at 22°C (closed circle) and B) TAG (closed circles) and solid fat content at 22 °C (open circles). Model parameters: A) X2 = 0.86, Y2 = 0.80, Q2 = 0.75, R2=0.75 B). X2 = 0.79, Y2 = 0.75, Q2 = 0.73, R2=0.75. Num = number of data points

at physiological temperature of lactating cows [16]. The present study demonstrate that milk fat is plentifully desaturated to secure fluidity in the mammary cells even in cows fed diets promoting saturated milk FA.

Correlations between melting off-set temperatures of milk fat, and FA and TAG compositions:

The influences of FA and TAG compositions on the thermal properties of milk fat were determined by PLS analysis. A PLS model in which FA was used as X variable and melting temperatures of the medium melting fraction of milk fat (MMT) as Y variable showed that 79% of the variation in FA (X2) could be used to explain 55% of variation in MMT (Y2). The predictive power of the model (Q2) as obtained through cross-validation was 51% with R2 of 0.38 (Figure 4a). Similarly, a PLS model between FA (X) and melting temperatures of the high melting fraction of milk fat (HMT) (Y) showed that 79% of the variation in FA (X2) could explain 52% of variation in HMT (Y2). From a cross validation, the predictive power of the model (Q2) was 49% and R2 of 0.39 (Figure 4b). HMT and MMT were positively related to major FA in milk fat such as C14 and C16. They were, however, negatively related to C18, C18:1 cis-9, C18:2 n6-cis, C18:3 n3, C18:1 trans-11 and CLA cis-9, trans-11.

FA such as C14 and C16 have high melting points (54.4 °C and 62.9 °C, respectively), and thus correlated positively to melting temperatures. This could be due to their incorporation into TAG which could have the ability to rise melting temperatures. Unsaturated FA such as C18:1 cis-9, C18:2 n6-cis and C18:3 n3 have negative melting points and therefore lowered melting temperatures. C18:0 contributed negatively to both melting off-set temperatures and crystallization onset temperatures. This is unexpected considering that C18:0 FA is saturated with a high melting point. In a study dealing with the seasonal variation in fatty acids composition and melting behavior of milk fat, a similar finding was observed for correlations between C18:0 and melting off-set temperatures of low melting fraction of milk fat [8]. C18:0 and C18:1 cis-9, which has been reported to lower melting temperatures of milk fat are closely related since C18 is used as a substrate for the formation of C18:1 cis-9 through the activity of stearoyl-coA desaturase [8]. Therefore, it may be logical that a negative correlation was observed between C18:0 and melting temperatures of milk fat. In the same way, though both C14:1 and C16:1 are unsaturated and could be argued to relate negatively to both HMT and MMT, they correlated positively to HMT and MMT.

Contrary to previous reports indicating that it might not be possible to affect HMT through feeding due to a requirement to maintain milk fat fluidity [8,9] the present study shows that it could be feasible to manipulate HMT through feeding albeit within physiological temperatures. Compared to the lipid supplemented groups, there was an increase of 3.5°C and 1.9°C in HMT and MMT, respectively, when milk fat from the lipid supplemented groups was compared to milk fat from the low fat diet (Table 1). Though the change in HMT is quite noticeable, there might be a limit on how much change is allowed, as it decides the level of crystallized fat in the range around cow body temperature. In this study, all HMT were below 39°C, the physiological temperature of cows, indicating that the desaturase mechanism together with the ratio between de novo synthesis and feed derived fat are very efficiently regulated when cow diets are manipulated.

Influence of FA and TAG on solid fat content (SFC) at 22°C:

Figure 5a & b shows the correlations between FA and TAG, and solid fat content at 22°C. A PLS model in which FA was used as X variable and solid fat at 22 °C as Y variable showed that 79% of the variation in FA could explain 75% of variation in SFC. The model had a predictive power of 73% and R2 of 0.75 (Figure 5a). Figure 5b shows a PLS model in which 55% of the variation in TAG (X) explained 31% of the variation in SFC at 22°C (Y). The predictive power of the model according to cross-validation was 75% with R2 of 0.80.

Positive correlations were observed between solid fat content and saturated FA such as C14 and C16. On the other hand, negative correlations were found between solid fat content and C18, C18:1 cis-9, C18:2 n6-cis, C18:3 n3, C18:1 trans-11 and CLA cis-9, trans-11 (Figure 5a). Regarding TAG, positive relationships could be found between TAGs C32, C34, C36, C42, C44, C46 and C48, and solid fat content. Negative correlations were detected between TAGs C40, C50, C52 and C54, and SFC (Figure 5b). These correlations were similar to those observed between FA, TAG and melting temperatures of the medium and high melting fractions of milk fat.

Conclusions

This study has demonstrated for the first time that is it possible to affect HMT of milk fat through low fat feeding which produce more low and medium chain FA. This is contrary to previous reports indicating that this is not possible due to a biological requirement to keep milk fat fluid at physiological temperatures (39°C) of cows [8,9]. However, the HMT were still under physiological temperatures of cows indicating that the biological mechanism secure full fluidity under most dietary manipulations. At the same time, significant solid fat contents were observed for milk fat from low fat feeding compared to milk fat from fat supplemented diets mainly due to increased production of short and medium chain FA. Therefore, from a nutritional and technological standpoint, low fat feeding could be a viable tool to manipulate the textural properties of milk fat products without detrimental health effects.

Milk Science International (71) 2018 P. 4-9
ISSN 2567-9538

Acknowledgement

We thank the Swedish University of Agricultural Sciences for the supply of milk samples. We further express gratitude to Dr. Bashar Amar, Department of Food Science, Aarhus University, for assistance in multivariate data analyses.

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