Study of the influence of cow breed on the fatty acid composition of milk fat and the characteristics of its melting

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Abstract. The article examines the influence of cow breed on the fatty acid composition of milk fat and on the characteristic indicators of milk fat melting in the form of the ratio of temperatures of thermal effects and the area under the melting curves obtained using the method of differential scanning calorimetry (DSC). Using two-factor analysis of variance, it was revealed that the breed significantly influenced the mass concentrations of fatty acids (FAs) C12:0, C14:1, C16:0. C18:0, C18:1 and the ratio of mass concentrations of fatty acids oleic (C18:1) to myristic (C14:0), stearic (C18:0) to lauric (C12:0) acid; oleic and linolenic to lauric, myristic, palmitic and stearic acid. At the same time, using multivariate regression analysis, it was revealed that individual indicators of fatty acid composition do not significantly affect the thermophysical parameters of melting, however, the total influence of some acids made it possible to obtain regression equations that quite accurately describe the experimental data, taking into account the relative scatter of the experimental data relative to the theoretically predicted ones.

1 Introduction

One of the most valuable components of cow's milk is milk fat. Its chemical composition is determined by the breed, season, lactation phase and diet of animals, etc.

To control the authenticity of milk fat, methods based on the analysis of the composition of fatty acids, the composition of triglycerides (TG), and the presence of phytosterols are traditionally used. However, a comprehensive chromatographic study in the form of component analysis is expensive, lengthy, and has a wide range of errors. Thus, when analyzing for phytosterols, the error (uncertainty) is ±20%, fatty acids and triglycerides ±3%.

Phase transitions during the melting of milk fat occur in a wide range of temperatures from -80 °C to 40 °C. They are influenced by various factors, such as the composition of fatty acids, triglycerides, polymorphism and size of milk fat globules, etc. [1]. The profile

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of differential scanning calorimetry thermograms of milk fat melting usually allows one to identify three endothermic peaks, caused by the presence of low-, medium- and high-melting fractions (LMPF, MMPF and HMPF, respectively) [2].

Since milk fat melts and crystallizes depending on temperature, this fact can be used to control the quality of milk fat. It has been established that the addition of 2% milk fat substitute to milk fat affects the differential scanning calorimetry melting profile [6], in particular, the ratio of melting temperatures t_3/t_2 on the differential scanning calorimetry graph (Figure 1).

However, it remains unknown how animal breed will influence fatty acid and triacylglyceride compositions, as well as the profile of differential scanning calorimetry curves during phase transitions.

2 Materials and methods

2.1 Object of research

The objects of research were milk fat obtained from the milk of cows of various breeds, feeding, housing, and seasons. The following samples were studied, presented inTable 1

Table 1. List of studied milk fat samples for further research.

Sample No.	Breed	Summer content	Winter content	Area	Locality
1	Red-motley	grazing in the meadows	stall	Ramonsky	Yamnoye village
2	Black and white	grazing in the meadows	stall	Ramonsky	Yamnoye village
3	Holstein	pasture	stall	Ramonsky	Yamnoye village
4	Red-motley (test sample)	pasture	stall	Novousmansky	village Novaya Usman
5	Jersey	grazing in the meadows	stall	Liskinsky	Dukhovoe village

2.2 Sample preparation

The milk was separated on a household separator "Rotor" at a temperature of 40-45 °C, producing cream and skim milk. The ratio of the volume of cream and the volume of skim milk (skimmed milk) is 1:7.5.

Butter was obtained by churning from cream cooled in a household refrigerator to 3 °C and then separating the butter from the buttermilk and washing it with ice water.

The separation of butter into fat and plasma was carried out according to GOST R 70238-2022 National Standard of the Russian Federation Milk and dairy products. Method for identifying the composition of the fat phase and determining the mass fraction of milk fat.

50 - 70 g of product were placed in a glass with a capacity of 150 cm^3. The glass with the product sample was placed in a thermostat and kept at a temperature of (55 +/- 5) °C until the product was separated into fat and milk plasma.

The upper fat fraction was separated by carefully pouring it into another glass and filtered through a dry pleated filter at the same temperature.

2.3 Measurements by differential scanning calorimetry

Differential scanning calorimetry (DSC) was carried out on an STA 449 f3 Jupiter simultaneous thermal analysis instrument.

The E-sensor was preliminarily calibrated by temperature and enthalpy taken from calibration substances (C10H16, C12H10, In, Bi, Zn).

A sample weighing 10 ± 1 mg in the molten state was placed in an aluminum crucible with a capacity of 30 μl, covered with an aluminum lid and placed in the sample cell on the sensor of the device.

Differential scanning calorimetry measurements were carried out according to the following temperature program:

- Heating from room temperature to 70 °C to completely melt the milk fat and remove the thermal history.
- Exothermic exposure at 70 °C to stabilize the device.
- Cooling at a rate of 5 K/min to a temperature of -170 °C for complete crystallization of the sample, as well as eliminating the influence of the inertial component of the device's furnace operation on the differential scanning calorimetry curve in the range from -80 °C and above.
- Isothermal exposure to stabilize the device.
- Heating to 60 °C at a speed of 5 K/min.

The system was cooled with liquid nitrogen using a CC300 system. The measurements were performed in an atmosphere of extremely pure helium (class 6) (purge gas flow rate – 50 ml/min, shielding gas flow rate – 10 ml/min).

2.4 Fatty acid composition

The fatty acid composition was studied by GLC in two different independent laboratories on various equipment in accordance with GOST R 52253-2004 "Butter and butter paste from cow's milk. General technical conditions": in the testing laboratory of the Federal Service for Surveillance on Consumer Rights Protection and Human Welfare, Federal budgetary health care institution "Center for Hygiene and Epidemiology in the Voronezh Region" (FBUZ) "Center for Hygiene and Epidemiology in the Voronezh Region, on the chromatograph "Crystal-2000 M" according to the regulatory documentation GOST 31663-2012 "Vegetable oils and animal fats. Determination by gas chromatography mass fraction of methyl esters of fatty acids", GOST 32261-2013 "Butter. Technical conditions"; at the Center for Collective Use of the Federal State Budgetary Educational Institution of Higher Education "VSU". Sample preparation was carried out according to GOST 32915-2014 "Milk and dairy products. Determination of the fatty acid composition of the fat phase using gas chromatography." Gas chromatographic analysis was carried out on an Agilent Technologies 7890B GC System chromatography-mass spectrometric complex with an Agilent Technologies 5977A MSD mass selective detector. The temperature of the sample injection unit is 270 °C, the analytical interface is 270 °C. Separation was carried out on a VF-23ms capillary column (20m x 0.150mm x 0.15 μm). The carrier gas flow rate is 1 ml/min, at a constant flow. The volume of the injected sample is 1 μl, flow division 40:1; temperature regime: 40 °C – isotherm 2 minutes, heating 10 °C/min, up to 120 °C, then heating at a rate of 5 °C/min to 230 °C, isotherm 3 minutes, then heating 10 °C/min up to 250 and isotherm 2 minutes. Electron impact ionization with a radiation energy of 70 eV was used. The signal was recorded using the total ion current (TIC) in the mass range 29-700 m/z. Data analysis and processing were carried out based on NIST11 databases (March 25, 2020), MassHunter v. software was used. B.06.00 and NIST MS Search 2.0. Correction factors for quantitative determination were introduced based on the analysis of a standard

mixture of fatty acid methyl esters Fatty Acid Methyl Esters Standard Mixture, Sigma-aldrich.

3 Results

3.1 Differential scanning calorimetry

The differential scanning calorimetry curves of the studied samples are presented in Figure 1.

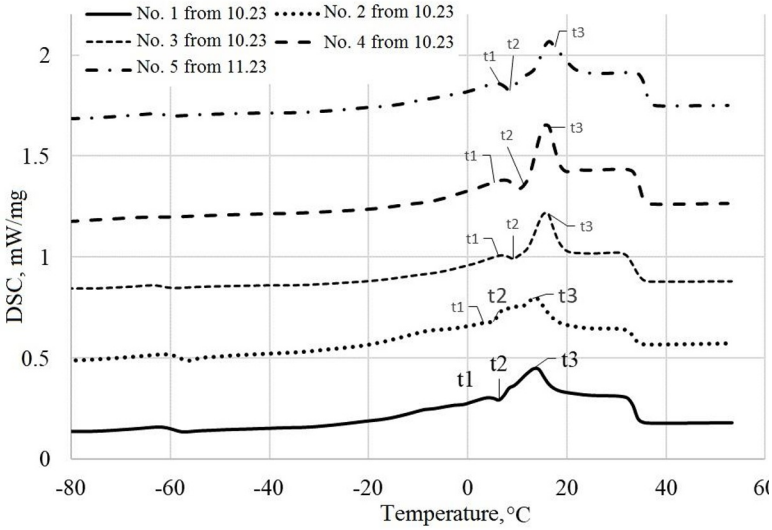

Fig. 1. Differential scanning calorimetry curves of milk fat samples.

In Fig. Figure 1 shows the main parameters characterizing the melting process: temperature of the first peak - t1; the trough temperature is t2, the third peak temperature is t3, and the area under the curve is S.

Melting parameters of milk fat samples are presented in Table 2

Figure 1 shows the main parameters characterizing the melting process: temperature of the first peak t_1; the trough temperature is t_2, the third peak temperature is t_3, and the area under the curve is S.

Melting parameters of milk fat samples are presented in Table 2

Table 2. Melting parameters of milk fat samples.

No.	t_2, °C	t_3, °C	t_3/t_2	S, J/g
1	12.3	16.7	1.36	88.7
2	9.6	16.4	1.71	93.2
3	10.1	16.2	1.60	94.4
4	10.1	17.9	1.77	92.9
5	8.6	18.0	2.09	93.2

3.2 Fatty acid composition

The results of the analysis of the fatty acid composition of milk fats in the Rospotrebnadzor laboratory are presented in Table 3.

Table 3. Mass fraction of fatty acid methyl esters in milk fat samples (Rospotrebnadzor Laboratory).

Fatty acids	Brief formulas	In English	Content of fatty acids according to GOST 32915-2014	No. 1 October	No. 3 October	No. 5 November	No. 2 October	No. 2 October Control sample
Oily	C4:0	Butanoic acid, methyl ester	2.4	2.5	3.2	3.1	3.3	2.6
Caproic acid	C6:0	Hexanoic acid, methyl ester	1.5	1.5	2.3	2.2	1.5	2.0
Caprylic acid	C8:0	Octanoic acid, methyl ester	0.9	0.8	1.4	1.2	0.7	1.3
Capric acid	C10:0	Decanoic acid, methyl ester	1.9	1.8	3.2	2.3	1.4	3.2
Decenoic acid	C10:1	4-Decenoic acid, methyl ester	0.2	0.3	0.4	0.3	0.2	0.3
Lauric acid	C12:0	Dodecanoic acid, methyl ester	2.4	2.3	3.7	2.5	1.7	4.2
Myristic acid	C14:0	Methyl tetradecanoate	8.7	9.5	11.5	9.8	6.8	13.0
Myristoleic acid	C14:1	Methyl myristoleate	0.9	1.3	1.3	0.9	0.7	1.3
Palmitic	C16:0	Hexadecanoic acid, methyl ester	25.5	26.4	33.1	28.2	24.8	38.2
Palmitoleic acid	C16:1*	9-Hexadecenoic acid, methyl ester, (Z)-	1.1	2.2	1.9	1.2	2.2	2.5
Stearic acid	C18:0	Methyl stearate	13.8	10.8	9.0	13.4	11.7	5.8
Oleic acid	C18:1*	9-Octadecenoic acid (Z)-, methyl ester	30.7	31.6	22.4	25.6	36.6	16.8
Linoleic acid	C18:2*	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	2.9	3.5	3.3	2.5	4.0	3.3
Linolenic acid	C18:3*	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1.5	1.0	0.4	0.5	0.9	1.0
Behenic Acid	C22:0	Docosanoic acid,		0.1	0.1	0.0	0.1	0.1
Arachidic acid	C20:0	Eicosanoic acid,		0.2	0.2	0.0	0.2	0.2
Linoleic acid (C18:2) to myristic acid (C14:0)				0.4	0.3	0.3	0.6	0.3
Oleic acid (C18:1) to myristic acid (C14:0)				3.3	1.9	2.6	5.4	1.3
Palmitic (C16:0) to lauric (C12:5)				11.5	8.9	11.3	14.6	9.1
Stearic acid (C18:0) to lauric acid (C12:0)				4.7	2.4	5.4	6.9	1.4
Oleic and linolenic to lauric, myristic, palmitic and stearic-1,3				0.7	0.4	0.6	0.9	0.3

The results of the analysis of the fatty acid composition of milk fats in the laboratory of the Center for Collective Use of the Federal State Budgetary Educational Institution of Higher Education "VSU" are presented in Table. 4.

Table 4. Mass fraction of methyl esters of fatty acids in milk fat samples (Laboratory of the Shared Use Center "VSU").

Fatty acids	Brief formulas	In English	Content of fatty acids according to GOST 32915-2014	No. 1 October	No. 3 October	No. 5 November	No. 2 October	No. 2 October Control sample
Oily	C4:0	Butanoic acid, methyl ester	2.4	2.9	2.4	4.0	3.5	2.1
Caproic acid	C6:0	Hexanoic acid, methyl ester	1.5	1.3	1.5	2.8	1.3	1.8
Caprylic acid	C8:0	Octanoic acid, methyl ester	0.9	0.9	1.1	1.5	1.0	1.4
Capric acid	C10:0	Decanoic acid, methyl ester	1.9	1.9	2.8	2.7	2.2	3.5
Decenoic acid	C10:1	4-Decenoic acid, methyl ester	0.2	0.3	0.3	0.4	0.2	0.4
Lauric acid	C12:0	Dodecanoic acid, methyl ester	2.4	2.6	3.6	3.1	1.9	4.7
Myristic acid	C14:0	Methyl tetradecanoate	8.7	9.8	12.0	11.0	8.0	13.1
Myristoleic acid	C14:1	Methyl myristoleate	0.9	2.0	1.7	1.4	1.1	1.9
Pentadecanoic acid	C15:0	Pentadecanoic acid, methyl ester	1.5	2.0	1.5	1.7	1.3	2.2
Palmitic	C16:0	Hexadecanoic acid, methyl ester	25.5	23.8	31.7	22.8	22.4	33.3
Palmitoleic acid	C16:1*	9-Hexadecenoic acid, methyl ester, (Z)-	1.1	1.9	1.9	1.7	2.0	2.1
Margaric acid	C17:0	Heptadecanoic acid, methyl ester	1.2	1.5	0.7	1.1	1.3	1.1
Stearic acid	C18:0	Methyl stearate	13.8	11.8	10.2	13.4	13.2	6.9
Oleic acid	C18:1	9-Octadecenoic acid (Z)-, methyl ester	30.7	25.5	20.5	21.3	29.9	15.5
Linoleic acid	C18:2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	2.9	2.0	2.1	3.4	2.7	2.6
Linolenic acid	C18:3	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1.5	0.8	0.3	0.6	0.8	0.9
Behenic Acid	C22:0	Docosanoic acid,		0.2	0.0	0.2	0.1	0.1
Arachidic acid	C20:0	Eicosanoic acid,		0.3	0.2	0.4	0.3	0.2
Linoleic acid (C18:2) to myristic acid (C14:0)			0.1-0.5	0.2	0.2	0.3	0.3	0.2
Oleic acid (C18:1) to myristic acid (C14:0)			1.6-3.0	2.6	1.7	1.9	3.7	1.2
Palmitic (C16:0) to lauric (C12:5)			5.8-14.5	9.1	8.8	7.4	11.8	7.1
Stearic acid (C18:0) to lauric acid (C12:0)			1.9-5.9	4.5	2.8	4.3	6.9	1.5
Oleic and linolenic to lauric, myristic, palmitic and stearic-1,3			0.4-0.7	0.5	0.4	0.4	0.7	0.3

4 Processing the results

4.1 Two-way ANOVA

Table 5 presents the factors, levels, and their designations for two-way ANOVA.

Table 5. Factors and levels of analysis.

Factors	Factor designation	Levels	Level designation
Cow breed	A	Red-and-white Black and white Holstein Jersey	1 2 3 5
Laboratory that performed the analysis	B	Rospotrebnadzor VSU Center for Collective Use	1 2

Based on the results of the analysis, the concentrations of fatty acids were identified, for which the laboratories gave results and which did not have significant differences, but at the same time the concentrations varied significantly depending on the breed: lauric acid (C12:0); stearic acid (C18:0); linolenic acid (C18:3) and their ratio: oleic (C18:1) to myristic (C14:0); stearic acid (C18:0) to lauric acid (C12:0); oleic (C18:1) and linolenic (C18:3) to lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0). Further, we will use only those concentrations of fatty acids and ratios, the measurement results of which did not depend on the laboratory in which the analysis was made, since other concentrations cannot be trusted, because laboratories give significantly different results. The reason for this may be random and systematic errors by laboratories in identifying some components as opposed to other components (Table 6).

Table 6. Influence of factors significantly influencing the concentrations of the following components in the biomaterial at a significance level of 0.05.

Fatty acid or ratio	Factor A Breed of experimental animals	Factor B Analysis results obtained in various laboratories
C12:0	+	-
C14:0	+	+
C14:1	+	+
C16:0	+	+
C18:0	+	-
C18:1*	+	+
C18:3*	+	-
Oleic acid (C18:1) to myristic acid (C14:0)	+	-
Stearic acid (C18:0) to lauric acid (C12:0)	+	-
Oleic (C18:1*) and Linolenic (C18:3*) to Lauric (C12:0), Myristic (C14:0), Palmitic (C16:0) and Stearic (C18:0)	+	-

All three of the above ratios varied significantly depending on the breed; further we will work with these ratios, since all identified concentrations of individual FAs are included in these ratios.

Using multivariate analysis of variance, it was not possible to identify high correlations of what is taken as a response (we take the following ratios of the parameters indicated in the table t_2, t_3, t_3/t_2, S). Those. each separately, for example S does not linearly depend on

the selected concentrations and ratios. Despite the fact that any response does not linearly depend on a factor, when we look for the dependence of this response on all factors simultaneously, this linearity appears.

Linearity manifests itself when the following ratios are taken as factors: oleic (C18:1) to myristic (C14:0); stearic acid (C18:0) to lauric acid (C12:0); oleic (C18:1) and linolenic (C18:3) to lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0).

Let's find the calibration equation according to Rospotrebnadzor data, i.e. dependence of S, t_2, t_3, t_3/t_2 on the ratio of the concentration of oleic (C18:1) to myristic (C14:0) acids; stearic acid (C18:0) to lauric acid (C12:0); oleic (C18:1) and linolenic (C18:3) to lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0), which we denote as X_1, X_2, X_3, respectively .

In what follows, we will use the least squares method, since we did not have the opportunity to normalize and encode the coefficients; We will do the calculation using matrices, since then we will not have to comply with the requirements that these data change with equal steps for all X_1, X_2, X_3, and we will obtain the equations:

$$S=b_0+b_1\cdot X_1+b_2\cdot X_2+b_3\cdot X_3=103.2+6.0\cdot X_1+2.1\ X_2-62.6\ X_3 \quad (1)$$

$$t_2=b_0+b_1\cdot X_1+b_2\cdot X_2+b_3\cdot X_3=5.5-2.0\cdot X_1-2{,}7\ X_2+37.4\ X_3 \quad (2)$$

$$t_3=b_0+b_1\cdot X_1+b_2\cdot X_2+b_3\cdot X_3=16.1-1.0\cdot X_1+0.9\ X_2-0.1\ X_3 \quad (3)$$

Thus, if we find the dependence of both S and t_2, t_3 t_3/t_2 separately, then equations (1)-(3) describe the melting process quite well.

If we check the calibration equations by substituting the ratios of the concentrations of fatty acids X_1, X_2, X_3, we will obtain the temperature parameters t_2, t_3 and area S, which differ from the experimental data with a relative error of a maximum of $9\bullet10^{-12}$%. The resulting regression equation quite accurately describes the experimental data according to the relative spread of the experimental data relative to the theoretically predicted ones. Due to the lack of initial samples for the values of S, t_2, t_3, when rounding the values of the regression coefficients, you should leave the same number of significant figures as in the values of S, t_2, t_3.

Next, solving a system of 3 three-factor equations, to check, we substitute into them the data from the laboratory of the Central Collective Use Center "VSU", and also substitute the data on the milk fat sample of breed No. 4, which did not participate in the training set, we obtain that the errors for the breeds that participated in the training set are errors less than 0.5%, and for a breed that did not participate in the training set, the error is less than 6%.

5 Conclusion

To understand whether the ratios of fatty acid concentrations fall under GOST R 52253-2004: oleic (C18:1) to myristic (C14:0); stearic acid (C18:0) to lauric acid (C12:0); oleic (C18:1) and linolenic (C18:3) to lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids, it is enough to obtain the parameters S using thermal analysis , t_2 and t_3 and substitute them into the equations. Thus, differential scanning calorimetry techniques can be used as reference methods for chromatographic analysis. A complex of chromatographic techniques and differential scanning calorimetry allows not only to confirm the naturalness of milk fat, but also with a high probability to distinguish the milk fat of some cow breeds from others.

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