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Anhydrous fat crystallization of ultrasonic treated goat milk: DSC and NMR relaxation studies

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ABSTRACT

Methods of NMR relaxation and differential scanning calorimetry (DSC) were used to study the crystallization of anhydrous milk fat (AMF) obtained from milk and subjected to ultrasonic (US) processing. Amongst the changes in the crystallization nature under the influence of ultrasound are the decrease in the crystallization temperature and the increase in the melting enthalpy of the anhydrous milk fat samples. The increase is ~30% at 20 min of isothermal crystallization and is presumably explained by the additional formation of β'-form crystals from the melt. The parameters of the Avrami equation applied to the description of experimental data show an increase in the crystallization rate in samples with ultrasonic treatment and a change in the dimension of crystallization with a change in melting temperature.

1. Introduction

Ultrasonic (US) processing of liquid food media allows solving numerous problems associated with increasing shelf life, improving the taste and technological properties of food products [1,2]. Recent works [3,4] on the study of US effects on goat milk indicated the agglomeration of fat globules after 30 min of sonication. Goat milk stands out among dairy products for its increased demand due to its anti-allergenic properties and special dietary digestibility. It is known that agglutinin is absent in goat milk, and it causes difficult clustering of the fat globules, which have a smaller size range (<3.5 μm) [5]. It leads to a low yield of butter and cheese. The ultrasonic processing of goat milk that results in the increase of fat globules size may help to solve the problem and increase the yield of goat butter and cheese during production. US treatment of goat milk may change the growth rate of of anhydrous milk fat (AMF) crystals, its temperature dependence, and the nature of the nucleation of crystals.

NMR relaxation and DSC methods are widely used to study the crystallization of milk fat (MF). Frederick et al. [6] proposed a new modification of the DSC method (stop and return), which allows one to evaluate the crystalline structure of fat sample at any time of isothermal crystallization. The crystallization of milk fat affects the texture, taste and rheological properties of products made from milk. These properties are very dependent on the speed and temperature during cooling, as well as the chemical composition and concentration of fatty acids. DSC and NMR relaxation methods were used by Smet et al. [7,8], to study the decrease in the crystallization temperature with a reduced content of unsaturated fatty acids in the MF. Bertram et al. [9] reported NMR relaxation molecular mobility with a change in the content of long-chain fatty acids in MF. DSC studies have shown that a decrease in the content of long-chain fatty acids causes a decrease in the crystallization temperature of MF. Investigating molecular mobility in a water-fat emulsion at different cooling rates, Panchal et al. [10] noted that the water content has little effect on the spin-spin relaxation times T_2 of the fat

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fraction and the nature of its crystallization. The study by NMR relaxation and X-ray diffraction proved that the mechanism of MF crystallization depends on the cooling rate. Wiking et al. [11] showed that rapid cooling gives two-stages crystallization, which was characterized by a polymorphic transition from the α to β crystal form. DSC and NMR studies of the whipping properties of MF by Wang et al. [25] showed that the optimal properties of the products, which were determined by the crystallization conditions, were achieved at temperatures of 4 °C − 7 °C. Comparison of the data obtained by DSC and NMR relaxation methods in the study of milk and dairy products were reviewed in articles by McCarthy [32] and Wahlgren & Drakenberg [33]. They note the differences in the data of the MF crystallinity obtained by these methods and their dependency on temperature. The DSC data exceeded the NMR values in the temperature range less than 20 °C and were the same for temperatures above 20 °C. This could be explained by the presence of an amorphous phase, which is predominantly present in the MF at low temperatures. From the NMR relaxation point of view, the viscosity of the liquid reduces the NMR values of the solid phase. The result may also be caused by the heterogeneous composition of triglycerides in the MF. Due to this, some of the crystals are more mobile and their properties are more similar to the liquid fraction than to the solid fraction. In addition, X-ray diffraction analysis (wide- and small-angle scattering) [11,12], rheological [12], spectral [13,14] and optical [14,15] techniques can help to understand both thermodynamic and structural changes caused by processing.

The majority (~98%) of milk fat consist of triacylglycerols (TAG), including a large number of esterified fatty acids [5]. TAG composition determines the nature of melting and crystallization. For goat milk, the distribution of TAG is unimodal with the maximum distribution over carbon atoms C40-C42, and about 65% of the total amount of TAG lies in the C36-C46 region [5,16]. Also, goat milk fat is distinguished by an increased content of short-chain fatty acids (C6: 0, C8: 0, C10: 0). All this affects the nature of the crystallization of goat fat. TAG, depending on external conditions, are capable of crystallizing into various polymorphic forms, differing in structure, melting point, and crystallization rate. There are 3 main polymorphic forms: α-form (hexagonal structure, unstable, low melting point), β'-form (orthorhombic structure, average melting point), β-form (triclinic structure, stable, high melting point) [17]. In milk fat, crystallization occurs in the α-form due to the low heat of crystallization and the high rate of nucleation [18]. The less stable α-form then transforms into more stable β' and β-forms, which have a great influence on the qualitative, rheological and structural properties of dairy products [10,19].

The free radicals arising under ultrasound effect on milk [20,21] can affect the chemical composition of fatty acids and TAG, thereby changing the crystallization conditions. The investigation of this change is the goal of our research. NMR relaxation and differential scanning calorimetry (DSC) were used to study the crystallization of anhydrous milk fat (AMF) obtained from milk subjected to ultrasonic (US) processing.

2. Materials and methods

2.1. Sample preparation

The goat milk used in this study was obtained from the Zaanen breed. It was stored at a temperature of +2 °C after milking. All experiments were performed within two days after milking. The milk composition was determined to be 3.8% protein, 4.3% fat, 4.4% lactose, and 86.9 % water. The separation of cream to obtain butter was carried out according to Russian Federation State Standards (ГОСТ Р 32261–2013). The milk fat obtained from butter was subjected to vacuum dehydration at 50 °C for 72 h. The water content in anhydrous milk fat (AMF) was found to be not >0.02%. Control samples were obtained from untreated (no sonication) milk.

2.2. Ultrasonication

The ultrasonic treatment was carried out using an UZO-150 "Avangard" unit (Russia) for 30 min at a temperature of 23 °C, on 2 L of goat milk. Pulse regime (55 pulses per minute,) bath-type sonoreactor with immersion US probe tip 50 mm diameter, 120 mm high. The operating frequency was 45 kHz, and the useful power output was determined calorimetrically using equation $P = mC\frac{dT}{dt}$, where m is the mass of the liquid, C is the specific heat, $\frac{dT}{dt}$is the change in the temperature of the liquid during the ultrasound exposure (120 s), and P = 12.3 ± 1.8 W. The sonication resulted in the agglomeration of fat globules in the milk. The agglomeration was determined using an OLIMPUS CX33 microscope with 1000 times magnification. The experiment was repeated 5 times for reproducibility check.

2.3. Determination of crystallinity by NMR relaxation

Isothermal crystallization of goat AMF samples was investigated using a MINISPEK PC-120 NMR relaxometer by an indirect method. Briefly, the AMF sample was heated to 50 °C, and held at this temperature for 20 min. The sample was then placed in a thermally insulated NMR sensor, where it was subjected to isothermal cooling. The magnetization decay amplitude of the sample, L_{70} (t) was measured at the time point, t = 70 μs from the beginning of the decay. Since the magnetization decay of crystalline protons is about 30–40 μs, the amplitude L_{70} (t) characterizes the protons of the molten liquid phase only. A decrease in the signal L_{70} (t) with time occurs due to isothermal (in this study, at 0 °C and 13.5 °C) crystallization in the sample, S (t) = [L_{70} (0) - L_{70} (t)]/L_{70} (0) - the relative content of the crystalline phase, where L_{70} (0) is the signal amplitude at 70 μs from a completely molten sample. The digitized magnetization decay curve was the average of 9 signal accumulations. The measurement time for each value L_{70} (t) was about 6 s. Temperature changes in the signal amplitude were calibrated using a sample with a known crystal content of 26.7%.

The obtained data of the curves of isothermal crystallization were approximated by the Avrami equation [22], using the least squares method.

$$ln\,U = -kt^n \quad (1)$$

where U is the liquid fraction of the sample; and k and n are Avrami's constant and exponent, respectively. In this study, U is given as U = L_{70} (t)/L_{70} (0). The Avrami equation is used to describe the processes of crystallization of palm oil fats [23,24], milk fat [13,13,25], and crystallization during the retrogradation of amylopectin in starch [26,27]. Avrami's model assumes that crystallization occurs because of the nucleation of crystallization centers and the growth of the crystals themselves. It assumes isothermal conditions for the formation of crystallization centers and the linear nature of the growth kinetics, in which the growth rate depends only on temperature, but not on time [28]. The parameter k is the crystallization rate constant, where both the nucleation rate and the crystal growth rate were taken into consideration. The parameter n, sometimes called the crystallization index, characterizes the mechanism of crystal growth. It depends on the time of crystal nucleation and characterizes the dimension in which the growth process takes place. These parameters can be determined from the linear dependence of log (-lnU) on logt or using the half-crystallization time, t_n = ln2/k.

2.4. Determination of the fatty acid composition of goat milk

The analysis of the fatty acid composition of the product was carried out using a Kristallux 4000 M gas chromatograph equipped with a flame ionization detector. The analysis was performed using a quartz column with the dimension of 100 m × 0.25 mm × 0.2 μm with a stationary phase FFAP. The mixture was identified using the SupelcoFAMEmix 37

components fatty acid methyl ester standard. Nitrogen was used as the carrier gas, with the following temperature separation program: column temperature T1 140 °C with 5 min exposure, T2 column 240 °C at 4 g/min; evaporation temperature 230 °C; the volume of the injected sample is 1 µl. The "NetChrom" software was used to control the analysis modes, record chromatograms, and process the obtained information.

2.5. DSk

The processes of nonisothermal crystallization and melting of goat fat were studied using a DSC204E Phoenix colorimeter (Germany) with the use of Proteus software. Temperature calibration was carried out for indium (156.6 °C) and mercury (−38.8 °C). The DSC peak deconvolution was performed using the NETZSCH Peak Separation program using the Fraser-Suzuki algorithm in treating asymmetric peaks. The melting thermogram experiments were carried out under the conditions as follows. The thermogram was heated up to 50 °C, and the temperature was maintained for 15 min. Then, the sample was cooled down to −60 °C at a rate of 20 °C per minute and maintained at this temperature for 15 min. Finally, it was heated up to 50 °C at a rate of 5 °C per minute.

The isothermal crystallization of fat samples was investigated by the stop and return method [6]. This method was carried out by interrupting the crystallization at various times. After heating the sample, the thermogram of the crystals melting formed during isothermal crystallization t_c was obtained. The experiment was carried out under the conditions as follows. The thermogram was heated up to 50 °C and the temperature was maintained for 15 min. It was then cooled at a rate of 20 °C per minute, until it reached the crystallization temperature, T_{cr}. The crystallization temperature in this study was 0 °C. The T_{cr} was maintained for a certain period of time, t_c. It was then heated up to 50 °C at a rate of 5 °C per minute. The cycle was repeated with a different time, t_c.

The obtained dependences of the change in enthalpy, ΔH on time t_c characterize the rate of crystallization. It should be noted that the quantity of crystals formed during the rapid cooling of the sample from 50 °C to T_{cr} was uncertain and is included in the final result as a constant value.

3. Results and discussion

3.1. Triacylglycerols and fatty acid composition of goat fat from saanen milk

The fatty acid goat milk compositions from the butter obtained in the presence and absence (control) of ultrasound are presented in Table 1. A comparison between the two samples shows that the fatty acid profile is unchanged ($P > 0.05$), indicating that there is no ultrasound effect on the chemical structure of fatty acids under the experimental conditions used. The contents of all studied acids are within the range reported in the literature [5,16]. Soares et al. [29], who studied the effect of ultrasound on goat milk at frequency of 25 kHz and power of 22 W/L, did not observe any effect of ultrasound on the chemical hydrolysis of goat fat. However, the enzymatic hydrolysis rate by lipase was found to increase upon ultrasound processing by 28% at 25 °C. This demonstrates the effect of ultrasound on lipase activity. In our study, 45 kHz ultrasound was used, with energy exposure of only ~6 W/L, resulting in the invariability of the fatty acid composition. The increase in the rate of enzymatic hydrolysis can also lead to the appearance of additional amounts of mono- and diacylglycerols, which affect the characteristics of fat crystals [14].

The content of fatty acids and triglycerides in goat milk largely determines the properties of dairy products, such as the melting point of the fat, the presence of a specific odor, and others. The determination of the composition of triglycerides and fatty acids makes it possible to predict these properties in many respects and, optimize them according to particular needs. Fig. 1 shows the distribution of the volumetric content of triglycerides depending on the carbon content for samples of

Table 1
Chromatographic profiles fatty acid composition obtained from goat's milk. The values are the mean average from three measurements performed with their standard errors.

Fatty acid name	Samples, vol %	
	Control	US experience samples
Alkanoic (saturated) acids		
Oil (C4:0)	1.90 ± 0,01	1.91 ± 0,02
Nylon (C6:0)	1.85 ± 0,04	1.90 ± 0,05
Caprylic (C8:0)	2.52 ± 0,06	2.52 ± 0,04
Capric (C10:0)	9.55 ± 0,03	9.57 ± 0,06
Lauric (C12:0)	5.36 ± 0,05	5.37 ± 0,05
Myristic (C14:0)	10.56 ± 0,07	10.58 ± 0,05
Tridecanoic (C13:0)	0.15 ± 0,02	0.16 ± 0,02
Pentadecane-cis	0.17 ± 0,03	0.18 ± 0,02
Pentadecanoic (C15:0)	0.75 ± 0,03	0.76 ± 0,04
Palmitic (16:0)	27.67 ± 0,43	27.62 ± 0,65
Margarine (C17:0)	0.63 ± 0,05	0.64 ± 0,07
Stearic acid (C18:0)	12.59 ± 0,55	12.68 ± 0,62
Arachidonic (C 20:0)	0.04 ± 0,01	0.04 ± 0,01
Geneicosan (C 21:0)	0.03 ± 0,02	0.03 ± 0,01
Behenic (C22:0)	0.07 ± 0,01	0.07 ± 0,02
Tricosodienic (C23:0)	0.003 ± 0.001	0.002 ± 0.001
Lignoeric (C24:0)	0.08 ± 0,02	0.09 ± 0,01
Monounsaturated fatty acids		
Myristic (C14:1)	0.12 ± 0,02	0.13 ± 0,01
Margarine-cis 10 (C 17:1)	0.16 ± 0,04	0.17 ± 0,02
Oleic (C18:1 cis)	18.20 ± 0,91	19.21 ± 0.74
Elaidinic (C18:1 trans)	2.03 ± 0,07	2.03 ± 0,05
Eicosane *cis*-11 (gondoinic) (C 20:1)	0.06 ± 0,01	0.06 ± 0,01
Selecholemic (C24:1)	0.09 ± 0,03	0.10 ± 0,02
Diene fatty acids		
Eicosadienic (C20:2)	0.70 ± 0,04	0.75 ± 0,07
Polyunsaturated fatty acids		
Linoleic acid (C18:2 cis)	2.78 ± 0,12	2.56 ± 0,19
Linolenic (C18:3 n3)	1.25 ± 0,08	1.27 ± 0,09
Gamma linoleic acid (C18:3 n6)	0.24 ± 0,03	0.24 ± 0,04
Arachidonic (C20:4 n6)	0.009 ± 0,002	0.011 ± 0,003
Docosahexaenoic (C22: 6 n3)	0.002 ± 0,001	0.003 ± 0,001
Eicosapentaenoic (C20:5 n3)	0.03 ± 0,01	0.03 ± 0,01
Dokosadienova (C 22:2)	0.008 ± 0,001	0.009 ± 0,001
Eicosatetraenoic *cis*-11,14,17 (C20:3 n3)	0.02 ± 0,006	0.03 ± 0,008
Eicosatetraene cis 8,11,14 (C20:3n6)	0.01 ± 0,002	0.02 ± 0,007

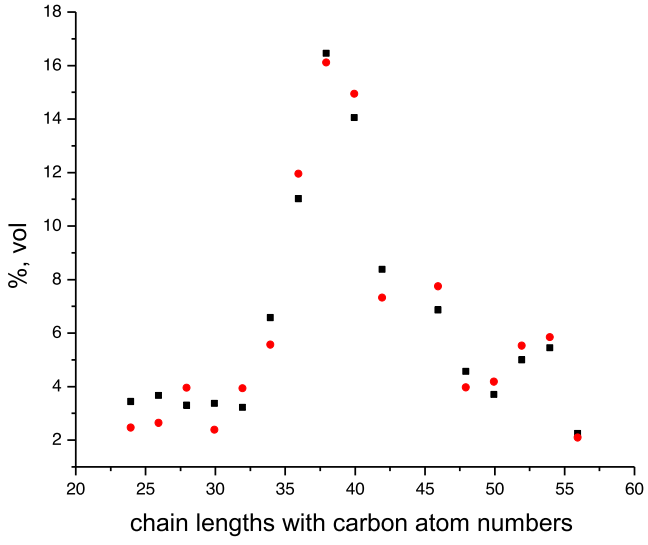

Fig. 1. Chromatographic profiles of goat milk fat triacylglycerols (TAG) of control (■) and US treated (●) milk samples. Data points are the average of three experiments.

goat milk subjected to ultrasound processing and without it.

The unimodal characteristic of TAG distribution with a maximum shown for carbon atoms C38 is slightly lower than that reported (C42) in the literature [5]. This may be due to the variation of the breeds and seasons. However, a comparison of fatty acids profiles between experimental and control samples did not reveal any difference ($P > 0.05$).

Various changes in milk under ultrasound influence, such as an initial decrease in fat globules and casein micelles size, agglomeration of casein micelles, and suppression of bacterial activity were observed mainly at lower frequencies of about 20 kHz and at higher power range [4,20] than in our experiment. In our previous study [30], we came to the conclusion that at low intensity, the effect of ultrasound has an effect at the macrolevel (agglomeration) without affecting the molecular composition and molecular mobility of fatty acids and triglycerides. This was evidenced by the absence of changes in the structure and nature of molecular movements (EPR studies) and changes in the diffusion nature of the movement of water molecules (NMR relaxation) samples of goat milk cream under ultrasound exposure. Therefore, the absence of changes in the composition of fatty acids and triglycerides under the action of ultrasound on goat milk samples can be explained by the low intensity of ultrasound and, possibly, a rather high frequency of 45 kHz. At this stage, technical capabilities did not allow us to determine the content of mono- and diacylglycerols in the total content of TAG, leaving this task for the next stage of study.

3.2. DSC studies of AMF crystallization and melting

The crystallization and melting thermograms of AMF samples in the absence and presence of ultrasound are shown in Figs. 2 and 3, respectively. Referring to Fig. 2, crystallization process of both samples begins at temperature around 11 °C. However, the crystallization peak maximum for the sample treated with ultrasonic is shifted towards lower temperatures, when compared to the control sample. This indicates an increase in the content of the fraction with a low crystallization temperature.

There had been numerous reports on the melting and crystallization of fats with various natures that note the features of the processes. These include a decrease in the melting point with an increased content of unsaturated fatty acids [7] and short-chain fatty acids [9], the dependence of the crystallization rate on the rate of high-low melting TAG [13,31], the dependence of crystallization characteristic (one or two step stage) on the change in the cooling rate [11] and on the temperature of isothermal crystallization [13].

The melting thermograms in Fig. 3 for both experiments in the absence and presence of ultrasound were found to be different from each other. The computer thermogram deconvolution of the control sample is shown in Fig. 4. The two characteristic peaks with maxima of ~11 °C and ~16 °C can be clearly seen. It was not possible to deconvolute the thermogram for an ultrasonic sample with the same program parameters. It indicates that the distribution by melting points in US experimental samples is unimodal. To understand the reason for this change, further analysis of the melting profiles was carried out. Based on the observed peaks from the decomposition curve in Fig. 4, we compare the isothermal crystallization of goat milk fat in the absence and presence of ultrasound at 13.5 °C, as shown in Fig. 5. At the temperature 13.5 °C the most part of the fraction, which has melting temperature ~11 °C remains at the melting state and should be excluded from the crystallization processes. Crystallization at 0 °C (Fig. 5) describes the behavior of both fractions.

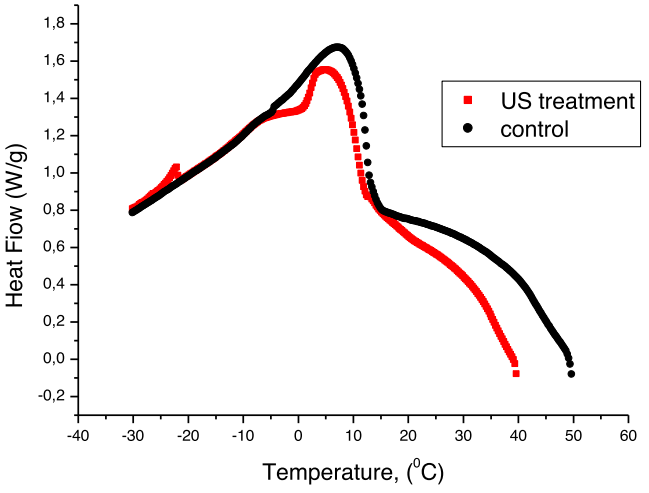

Fig. 2. Crystallization curves of goat milk fat samples from DSC experiment. The heating rate was at 20 °C/min.

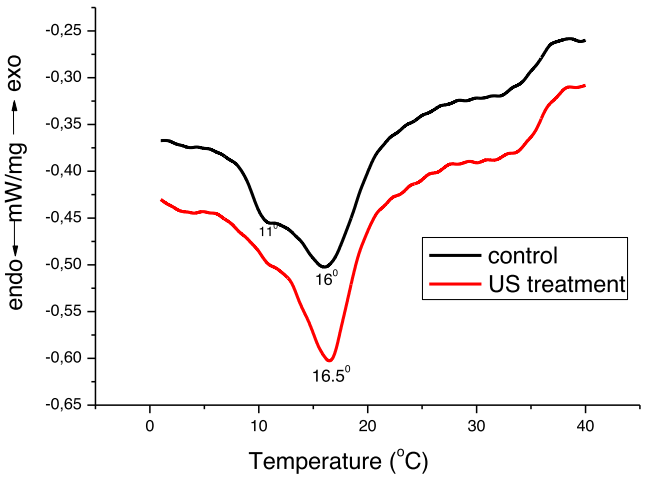

Fig. 3. Melting curves of goat milk fat samples from DSC experiment. The heating rate was at 5 °C/min.

3.3. Isothermal crystallization. NMR relaxation studies

The isothermal crystallization study was carried out by the magnetic relaxation method as described before. The crystallization curve at 13.5 °C (Fig. 5) is characterized by the presence of an induction period for 25–30 min and a sigmoid shape, which is typical for crystallization from a melt into one polymorphic form [13]. However, there was no difference found between the samples with and without ultrasonic treatment. This indicates the similarity of the crystallization processes of the fraction with an increased melting point in these samples.

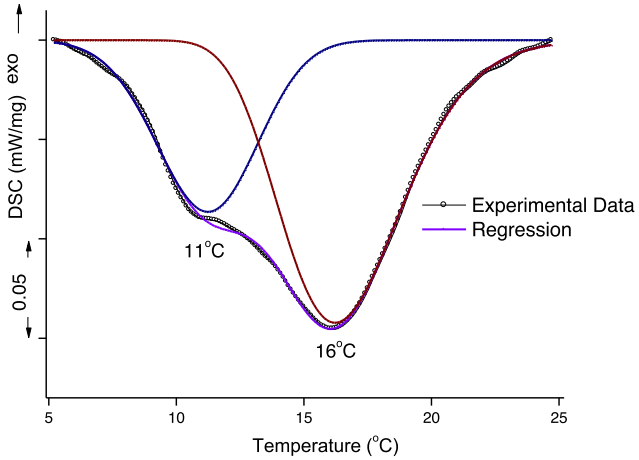

Fig. 4. DSC control melting curve deconvolution.

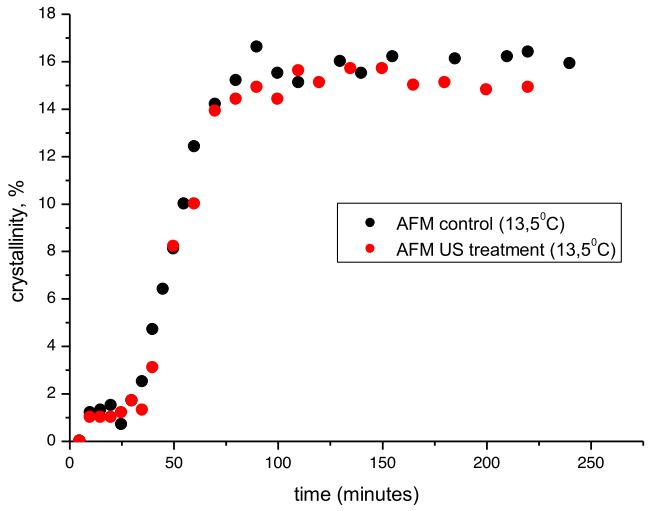

Fig. 5. Crystallinity as a function of time during isothermal crystallization for AMF samples of US treatment and control milk. Crystallization temperature 13.5 °C.

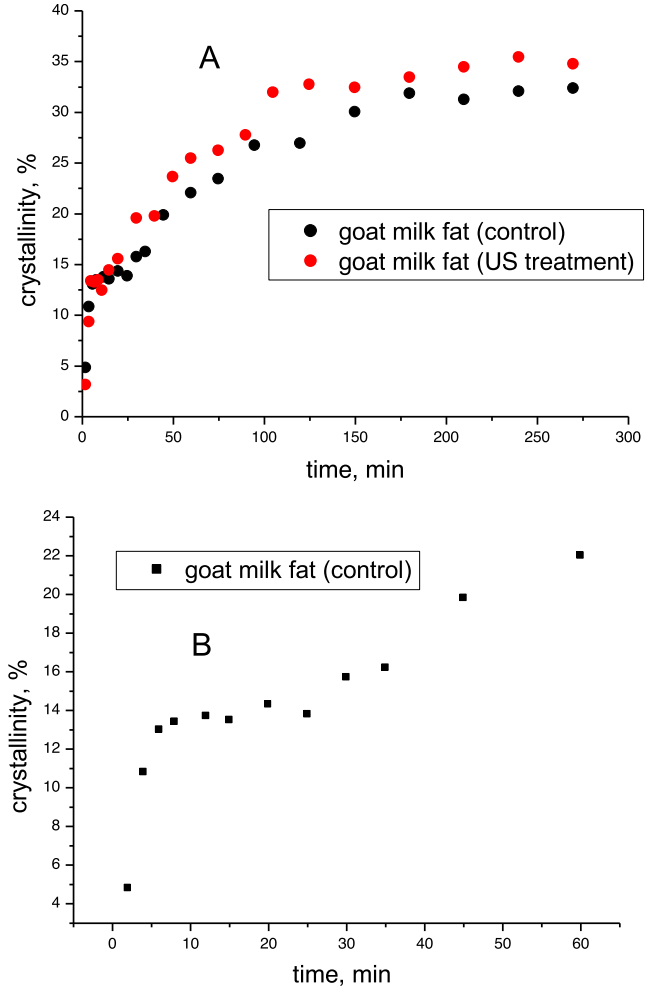

Fig. 6. (A). Crystallinity as a function of time during isothermal crystallization for AMF samples of US treatment and control milk. (B) is increased plateau region. Crystallization temperature 0 °C.

The crystallization curves at 0 °C is shown in Fig. 6(A). The absence of an induction period and certain differences between the ultrasound experiment and the control can clearly be seen. The crystallization curve of AMF with ultrasonic treatment shows a constant increase in the crystalline phase, while the curve of the control samples is characterized by the presence of a constant plateau in the period from 7 to 25 min (Fig. 6B). Many studies describe such a plateau as a two-stages crystallization, when crystals of the unstable α-form formed from the melt transform into the stable β'-form. [7,13].

3.4. Isothermal crystallization. DSK stop & return method

To further understand the effect of sonication, the isothermal crystallization DSC experiments in the presence and absence of ultrasound were conducted by the stop & return method. Fig. 7 shows the melting thermograms of rapidly cooled control samples of AMF, where isothermal crystallization at 0 °C was interrupted after 10, 15, 20, and 40 min. To be consistent with the literature [11,25,32], the constituents of fat fractions corresponding to low- medium- and high-temperature regions, are labelled as peaks 1, 2, and 3 in our experiment. An increase in the crystallization time is characterized by the change in the shape of peaks 1 and 2. During 10–20 min, the area of peak 1 decreases, the area of peak 2 increases and the area of peak 3 is constant. In this case, the sum of the areas of peaks 1 and 2, which characterizes their total enthalpy of melting ΔH, does not change (Table 2). This leads us to the speculation that peaks 1 and 2 characterize polymorphic forms of fat crystals α and β', respectively, and their mutual changes - the transition from one form to another. The constant total enthalpy of melting of these two peaks is consistent with the trend observed in the crystallization curve (Fig. 6) over a period of 7 to 25 min.

After 40 min of crystallization, peak 1 is found to almost disappear, whilst the areas of peaks 2 and 3 are found to increase. This indicates that crystallization occurs predominantly in the β'-form. The maxima of peaks 1 and 2 are shifted towards lower temperatures. This indicates that fractions with a high melting point crystallize first [6]. Similar DSC studies carried out with AMF samples from ultrasonically treated milk showed a similar pattern of the location of peaks 1, 2, and 3 on the melting thermograms (thermograms are not shown), however, the time change in the enthalpy of melting ΔH of peaks 1 and 2 had a different character.

The total value of ΔH, where ΔH is the area sum of peaks 1 and 2 were found to increase with the crystallization time in the period of 10–20 min for the sonicated samples compared to those of control samples (Table 2). This increase is about 30 % of the total area of peaks 1

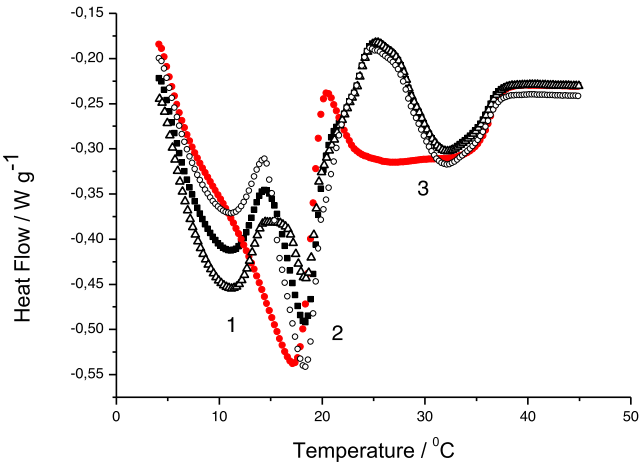

Fig. 7. Melting curves of the stop and return method. The isothermal crystallization of goat milk fat was interrupted at 10 (Δ), 15(■), 20 (○) and 40 (●) minutes as measured by DSC.

Table 2
Melting enthalpy change (ΔH) of endothermic peaks 1, 2 and 3 (Fig. 3) for control and US treatment goat AMF samples in stop and return experiment. An average of three independent measurements is provided.

	Time of crystallization, minutes	Melting enthalpy ΔH of two endothermic peaks (1 + 2), J/g	Melting enthalpy ΔH of endothermic peak 3 J/g
control	10	35,3 ± 0,9	9,3 ± 0,5
	15	35,1 ± 1,1	9,7 ± 0,4
	20	36,5 ± 0,8	10,1 ± 1,2
US treatment	10	34,5 ± 1,0	10,3 ± 1.9
	15	39,1 ± 0,9	11,2 ± 1,8
	20	46,3 ± 1,2	10,8 ± 1,5

and 2 in the control. This is mainly contributed by the increase in the area of peak 2. The areas of peaks 1 in the samples of the ultrasonic experiment and control for the same crystallization times were approximately equal (data not shown). If we assume the same rate of nucleation of α crystals and their transition to the β'-form, then an increase in the enthalpy of melting of peaks 1 and 2 indicates an additional increase in β'-crystals, which can be formed directly from the melt. The change in peak 3 was similar to the changes in the control sample, which confirms the absence of differences in the experiment and control for the crystallization curves of fractions with a higher crystallization temperature (Fig. 5). As shown in a number of studies [13,23,25], the formation of α and β' forms of crystals is largely determined by the difference between the crystallization temperature T_{cr} and the cooling temperature T_{cool} (degree of supercooling). At a large temperature difference (or high speed cooling), a two-stage crystallization is observed. First, an unstable α form is formed, which then transforms into a more stable β' form. A small temperature difference is characteristic of the formation of the β form from the melt. When, upon cooling, first of all, high-temperature fractions crystallize (which is characterized by a shift of the enthalpy peaks towards lower temperatures [6]), the temperature T_{cr} of the remaining part of the melt decreases. At a certain stage, the difference between T_{cr} and T_{cool} reaches values at which crystallization into the β' form occurs from the melt. An increase in the rate of lipase hydrolysis observed during ultrasonic processing of goat milk [29] can lead to an increase in the content of di- and monoglycerides, which in turn can reduce T_{cr} and lead to additional formation of β' crystals from the melt. The nature of polymorphic transitions during isothermal crystallization is confirmed in various studies [11,13,25]. Further justification can be obtained from x-ray diffraction studies, which will be part of our future study.

3.5. Approximation of crystallization curves by the Avrami equation

To mathematically describe the isothermal crystallization upon time, the Avrami equation was used. The Avrami equation is shown in Eq. (2) below.

$$log(-lnU) = log(k) + n log(t) \quad (2)$$

where U is the melt fraction in the sample, and the parameters k (Avrami constant) and exponent n characterize the crystallization process.

Fig. 8 shows the approximation of the isothermal crystallization curves at 0 °C by the Avrami fitting. The k and n values were successfully calculated due to the linear dependence of log (-lnU) on log (t). The results are presented in Table 3. Note that the effect of the crystallization temperature on the value of k decreases by 4 orders of magnitude when the temperature changes from 0 °C to 13.5 °C. Previous reports [13,13,25] have shown that when changing the crystallization temperature of milk fat within 5 °C – 25 °C, the value of k varied from 10^{-1} to 10^{-8} showing that crystallization proceeds slower at elevated temperatures due to slower nucleation and growth rates.

Thus, by analysizing the k values for experiments in the presence and absence of ultrasound at a crystallization temperature of 0 °C, it can be argued that the ultrasonic effect significantly increases the crystallization rate ($P < 0.05$). Since the crystallization rates of the high-

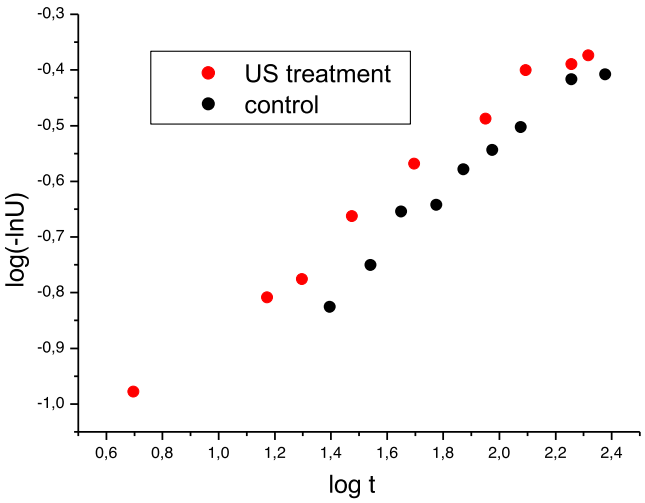

Fig. 8. Fitting the curves of isothermal crystallization at 0 °C (taken from Fig. 6) by the Avrami equation.

Table 3
Avrami constants k, Avrami exponents n and coefficient of determination R^2 from Avrami equation [log(-lnU) = logk + nlogt] isothermal crystallization fitting, where U is the liquid fraction ratio.

	k, min^{-n}	n	R^2
Control, 13,5°C	$5,28*10^{-6}$ $\pm 1,43*10^{-6}$	2,73 ± 0,64	0,982
US treatment, 13,5°C	$6,31*10^{-6}$ $\pm 0,95*10^{-6}$	2,61 ± 0,38	0,974
Control, 0 °C	0,03 ± 0,01	0,9 ± 0,3	0,967
US treatment, 0 °C	0,09 ± 0,03	0,5 ± 0,2	0,988

temperature fraction of AMF in the ultrasonic experiment and control at 13.5 °C are very small and practically indistinguishable, it can be assumed that the change in k in the ultrasonic experiment at 0 °C occurs due to a change in the profile of the low-temperature fractions. As it was mentioned above US treatment of milk increases the enzymatic glycolysis rate [29]. This may lead to the increace in the mono- and diacylglycerols content, which in its turn change the profile of the low-temperature fractions, lower the crystallization temperature and increase the crystallization rate [14].

The parameter n, which, according to Avrami's theory, characterizes the dimensionality of crystal growth, also changes when the crystallization temperature changes (Table 3). As noted in [25], an increase in n from 1 to 4 characterizes the transition of crystallization from linearity to multidimensionality. As noted in [14], n is the phenomenological index of crystallization, in which the limitations of Avrami's theory are manifested, determined by the possibility of nucleation observing and determining the nature of crystallization. Changes in n observed with an increase in the crystallization temperature (Table 3) allow only a qualitative assessment of the transition from one-dimensionality (n = 0.5–0.9) to multidimensionality (n = 2.6–2.7) of the nature of

crystallization. The difference in n values for ultrasonic experience and control at a crystallization temperature of 0 °C cannot serve as a quantitative assessment of ultrasonic exposure.

4. Conclusions

Our investigation on the effect of ultrasound on goat milk samples revealed the following key observations. No changes in the quantitative composition of triacylglycerols and fatty acids were found. Isothermal crystallization of anhydrous goat milk fat, studied by the NMR relaxation method, showed a change in the nature of crystallization during ultrasonic treatment. The experimental curve for a low crystallization temperature of 0 °C showed the constant increase in the crystal content, while the control curve was characterized by the presence of a plateau. DSC studies using the stop & return method showed that such changes can occur due to the additional formation of β'-form crystals from the melt. Crystallization curves at a temperature of 13.5 °C for the experiment and control did not differ, and this led to the speculation that changes occur in the fraction with a low melting point. This assumption is confirmed by the data of DSC studies of nonisothermal crystallization, which show a reduced maximum of the crystallization temperature in the experiment with ultrasound exposure in comparison with the control. Such a decrease may indicate an increase in the fraction with a low melting point (mono- and diacylglycerols), however, to finally confirm these assumptions, additional studies are required (study of the composition of mono- and diacylglycerols and X-ray diffraction studies). Approximation of the experimental data by the Avrami equation allows us to conclude that there is an increase crystallization rate in samples with ultrasonic treatment.

CRediT authorship contribution statement

Andrey Sergeev: Conceptualization, Investigation, Writing - review & editing. **Natalia Shilkina:** Investigation, Methodology. **Mikhail Motyakin:** Conceptualization, Investigation, Writing - original draft. **Irina Barashkova:** Methodology, Investigation. **Victoria Zaborova:** Writing - review & editing. **Ksenia Kanina:** Investigation. **Nina Dunchenko:** Resources. **Olga Krasulya:** Conceptualization, Resources, Writing - review & editing. **Nor Saadah M. Yusof:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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