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EPR and NMR study of molecular components mobility and organization in goat milk under ultrasound treatment

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ABSTRACT

The effect of ultrasound treatment on molecular mobility and organization of the main components in raw goat milk was studied by EPR and NMR spectroscopies. NMR relaxation studies showed an increase in the spin–lattice T_1 and spin–spin T_2 relaxation times in goat milk products (cream, anhydrous fat) and change in the diffusion of proton-containing molecules during ultrasound treatment. The diffusion became more uniform and could be rather accurately approximated by one effective diffusion coefficient D_{eff}, which indicates homogenization of goat milk components, dispersion of globular and supermicellar formations under sonication. EPR studies have shown that molecular mobility and organization of hydrophobic regions in goat milk are similar to those observed in micellar formations of surfactants with a hydrocarbon chain length C12–C16. Ultrasound treatment did not affect submicellar and protein globule organization. Free radicals arising under ultrasound impact of milk reacted quickly with components of goat milk (triglycerides, proteins, fatty acids) and were not observed by spin trapping method.

1. Introduction

Ultrasonic effect on food and food product is manifested as its homogenizing, antibacterial, changes in the chemical composition and many more. Amongst the factor is due to the action of free radicals generated during cavitation [1] Such a phenomenon as cavitation, which appears during the passage of an ultrasound wave, consists in the formation of pulsating gas bubbles in the liquid at collapse of which temperature ~ 10^3–10^4 K and pressure ~ 10^2 mPa [2]. These critical conditions lead to the formation of free radicals as well as subsequent structural and chemical transformations. Amongst the methods for detecting such transformations are the nuclear magnetic relaxation (NMR) and electron paramagnetic radio spectroscopy (EPR) [3–5].

The NMR relaxation technique is widely applied in the study of chemical transformations, internal structural rearrangements, qualitative changes in food products under various technological methods of exposure and many more [6]. Estimation of the molecular mobility of proton-containing molecules in milk by the magnetic relaxation method is rather difficult due to the complexity of the interpretation of the data obtained. Milk components of various composition (water, triglycerides, fatty acids, proteins) and structure (fat globules, casein micelles, solutions of proteins, sugars, minerals) provide wide, yet often overlapped spectra of magnetic relaxation characteristics. These characteristics include the spin–lattice (T_1) and the spin–spin (T_2) relaxation times, and the self-diffusion coefficient of proton-containing molecules D, determined by the method of a pulsed field magnetic gradient. The literature contains a large number of works on the study of relaxation times [3,7,8] and diffusion in milk and dairy products [9,10]. The results of these studies shown that the relaxation times of water protons and fat protons overlap to a large extent, which introduces certain difficulties in measuring the diffusion coefficient. The wide distribution of these parameters often forces the use of averaged, “effective” values of these parameters.

The second method applied in this study for the characterization of

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molecular mobility in goat milk is EPR spectroscopy. Particularly, EPR methods of spin probe and spin trap. EPR spectroscopy of spin probes is an elegant method for studying complex heterogeneous system. The magnetic resonance parameters of the EPR spectra of spin probes provide information on the molecular organization of the system under study, its local mobility and polarity of spin probe environment [11–13]. The localization of spin probes in molecular system depends on their chemical structure. Therefore, the use of different probe makes it possible to obtain the necessary information about different regions of the heterogeneous system. The main parameters of the EPR spectra analyzed in this case are the correlation time of the spin probe rotation, τ, and the hyperfine coupling constant (hcc), a^N. The correlation time of a spin probe is inversely proportional to the frequency of its molecular motions; thus, it allows one to estimate the local mobility and molecular organization at the location of the spin probe. The hcc constant is proportional to the spin density of the unpaired electron on the nitrogen atom, which depends on polarity of the solvent. It is well known [11] that the nitroxyl group has two resonance structures: neutral and zwitterionic as shown in Fig. 1. In polar solvents, the equilibrium is shifted towards the zwitterionic nitroxyl structure, in which more spin density is concentrated on the nitrogen atom, and, accordingly, the hcc constant of the spin probe is higher.

EPR method of spin trap is widely used to detect short-living radicals arising in various systems [14,15], including food products [5,16,17]. The method is based on the reaction of a short-living radical R• with a spin trap leading to the formation of a stable nitroxyl radical (spin adduct) as shown in Fig. 2. In this work, 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was used as spin trap. The nature of the trapped short-living radical is determined from the magnetic resonance parameters of the formed nitroxyl radical [18]. Note that the short-living radical R• can also react with a nitroxyl radical forming a diamagnetic molecule. Though this reaction is not major, but its occurrence in solution could affect the lifetime of the DMPO spin adduct.

Casein is the main protein in milk. The main part of casein is contained in the form of micelles – colloidal particles which are associates of main fraction of casein. Casein micelles are spherical, hydrated particles with a diameter of 50–600 nm [19]. Micelles are composed of submicelles – the many spherical micelle particles ranging about 10–20 nm in size [19]. Submicelles are connected to each other by colloidal phosphate, hydrogen and other bonds. The main forces promoting the formation and stabilization of casein submicelles are the hydrophobic interaction of its water hating parts. To date, scientists and researchers in the area have proposed several models of casein micelles [19–22]. Most authors believe that casein micelles consist of densely packed submicelles built according to the core–shell type. Thus, the whole milk as an object of study can be considered as a complex heterogeneous system. The hydrophilic area of localization of the probe can be determined mainly by an aqueous solution of salts and lactose, partly by the surface of casein micelles, and hydrophobic – by the inner part of micelles and fat globules. In this study, the exact location of the spin probes is tough to be specifically identified, however it can be well predicted according to the concept of "hydrophilic and hydrophobic areas" on the micelle system.

Goat milk is becoming a popular option amongst the many dairy products due to the anti-allergenic properties and special dietary digestibility. Goat milk also possesses specific properties of such as smaller size of fat globules and low content of β-casein. This makes it difficult to obtain butter from goat milk. In [23], it was shown that ultrasonic processing of goat milk (~40 kHz, ~ 10 W) for 30 min leads to a significant decrease in the level of microflora and an increase in the size of fat globules. This in turns facilitates the production of butter. In some other reports [23–26], an initial decrease in the size of fat globules and casein particles during ultrasound treatment of milk with subsequent agglomeration after 30 min of ultrasound exposure was observed. These data served as basis during the design of the conditions in our experiment. The experiments were carried out with aim to determine the effect of ultrasound exposure on the molecular mobility and molecular organization of the main components of raw goat milk using EPR and NMR spectroscopy.

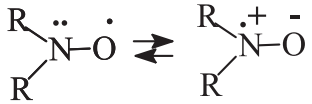


Fig. 1. Resonance structures of nitroxyl group.

2. Materials and methods

2.1. Sample preparation

Goat milk used in this study was obtained from the Zaanen breed. It was stored after milking at temperature of 0-2°C. All experiments were then performed within the next two days. The milk composition was characterized to be 3.8% protein, 4.3% fat, 4.4% lactose, and 86.9% water content. Separation of cream and obtaining butter was carried out according to Russian Federation State Standards (ГОСТ Р 32261-2013). 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 more than 0.02%. The control samples were obtained from the untreated milk.

2.2. Ultrasonication

Ultrasonic processing of milk (500 ml) was carried out in the thermostatic ultrasonic bath Elmasonic (Germany) for 30 min at a temperature of 23°C. The operating frequency is 37 kHz and the useful power output was determined calorimetrically using the 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 60 s of ultrasonic action. The P was calculated to be 22.3 ± 1.4 W.

2.3. NMR relaxation

The proton relaxation time investigation of the dairy products were carried out on a Minispec PC-120 Brucker (Germany) operating at a frequency of 20 MHz. The measurements were carried out at 40 °C. The spin–lattice relaxation time (T_1) was determined using a pulse sequence of 180°–90° (inversion-recovery) under the following conditions: 90° pulse duration of 2.3 μs, with 20 points on the curve, and delay between scans to be 4–10 s. The spin–spin relaxation time (T_2) of protons was determined using the Carr-Purcell-Mebium-Gill (CPMG) pulse sequence under the following conditions: 90° pulse duration of 2.3 μs, τ - time between 90° and 180° pulses to be 150 μs, and 1500 points on the decay curve. Accumulation of 9 scans were carried out with delay between scans to be 4–10 s. The obtained curves of changes in magnetization were analyzed using the discrete multi-exponential decomposition program MULTIT1a and MULTIT2a (Brooker), or the curves were digitized with a frequency 1 MHz and the ORIGIN 9 program was used. The relative error of relaxation measurements did not exceed 6%.

The diffusion coefficient, D of protons was determined on a Minispec PC-120 Brucker (Germany) with two-pulse sequence pulsed gradient unit [39]. The D values were obtained from the dependence (1) of the relative amplitude of the spin echo on the parameters of the pulsed magnetic field gradient.

$$ln(A_n/A_0) = \gamma^2 g^2 \delta^2 (\Delta - \delta/3) D \quad (1)$$

where A_n and A_0 are the echo amplitudes in the presence and absence of a pulse gradient, γ is the gyromagnetic ratio for a proton (26.75 * 10^7rad. $T^{-1}sec^{-1}$), g and δ are the amplitude and duration of the pulse gradient, Δ is the time between pulse gradients (time of diffusion), D is

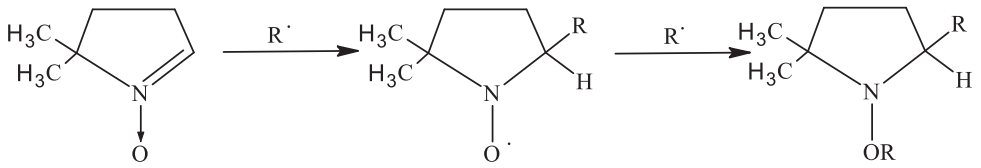


Fig. 2. Structure of DMPO spin trap and chemical reactions involving the spin trap.

the diffusion coefficient. Range of variation g = 0.4–2.5 Tm^{-1}. The variable parameters in the measurements were Δ and g, $\delta = 500$ μs was constant. The calibration of the impulse gradient g was carried out using the diffusion coefficient of water at 25° C ($D = 2.3 * 10^{-9}$ m^2 s^{-1})

2.4. EPR experiment

The spin probes 2,2,6,6-Tetramethylpiperidin-1-oxyl (TEMPO), 5-DOXYL-stearic acid(5-DSA), and 16-DOXYL-stearic acid(16-DSA) were purchased from Sigma-Aldrich; 4-Trimethylammonium-2,2,6,6-tetramethylpiperidine-1-oxyliodide (CAT-1) from Molecular Probes, US; and spin trap 5,5-Dimethyl-1-pyrrolineN-oxide (DMPO) from Abcam, UK were used in the work. The structures of the chemicals are presented below.

The spin probes were introduced in the samples as the following way. Stock solutions of the spin probes TEMPO, 5-DSA and 16-DSA radicals were prepared in ethanol; and CAT-1 radical in distilled water. The solutions were then divided into several vials, and the solution in the vials was evaporated at room temperature. The resultant spin layer was dissolved in goat milk or cream and stirred for 30 min. Radical concentration in the samples did not exceed 2×10^{-4} M.

Stock solution of DMPO spin trap was prepared in goat milk. Concentration of DMPO was 62 mM. All studied solutions were placed in EPR glass capillaries and sonicated for 30 min in a sonication bath.

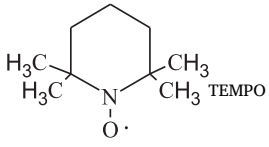
X-band EPR spectra were recorded on a Bruker EMX (Germany) spectrometer operating at 9.7 GHz and 100 kHz magnetic field modulation. The spectral acquisition was carried out at temperature 293 K; microwave power 2.1 mW; modulation amplitude below 1 G; sweep width 100 G and with 1024 points recorded. WINEPR and SIMFONIA (Bruker) programs were used for the mathematical analysis of EPR spectra. To determine the correlation time of radical rotation, experimental spectra were simulated using a program developed by Freed and colleagues [27]. Model of isotropic Brownian rotation was referred to. Magnetic resonance parameters of radicals used in simulations are presented in articles [11–13,28–37].

3. Results and discussion

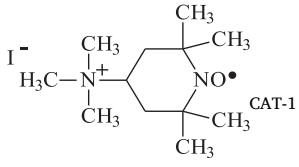
3.1. EPR spectroscopy

The EPR study result of the radicals in goat milk or cream are presented as Fig. 3 and is also summarized in Table 1.

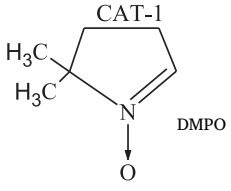
In heterogeneous systems, the TEMPO radical localized predominantly in regions with lower molecular density and a sufficiently large free volume for the probe rotation. These regions can be both hydrophobic and hydrophilic [13,33]. Spectrum 1a in Fig. 3 presents the EPR spectrum of the TEMPO radical in a goat milk sample. The spectrum is a superposition of two signals from radicals localized in morphologically different molecular regions. In Fig. 3 and Table 1, lines and numbers related to different signals are marked with "*" and "o". The simulation of spectrum made it possible to obtain the correlation times of the probe rotation, τ, and isotropic hcc constants, a^N, (Table 1). Comparison of the calculated hcc constants of the TEMPO radical (they reflect the polarity of the local radical environment) with those known in literature [33] suggests that the first radical (the spectrum marked with "o" in Fig. 3) is localized in the hydrophilic region of the studied system (aqueous medium), while the second radical (the spectrum marked with "*" in Fig. 3) is localized in the hydrophobic part of it. The obtained result confirms the presence of hydrophobic and hydrophilic regions in milk solution and shows the possibility of controlling their percentage using nitroxyl radical. This can be confirmed by the spectrum of the TEMPO radical in goat milk cream (spectrum 1b in Fig. 3). As can be seen from this spectrum, the signal from radicals in the hydrophilic region noticeably decreased which indicates a decrease in the percentage of the aqueous



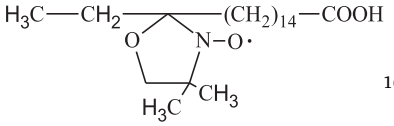
TEMPO



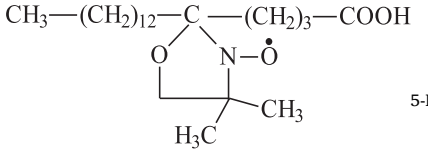
CAT-1



DMPO



16-DSA



5-DSA

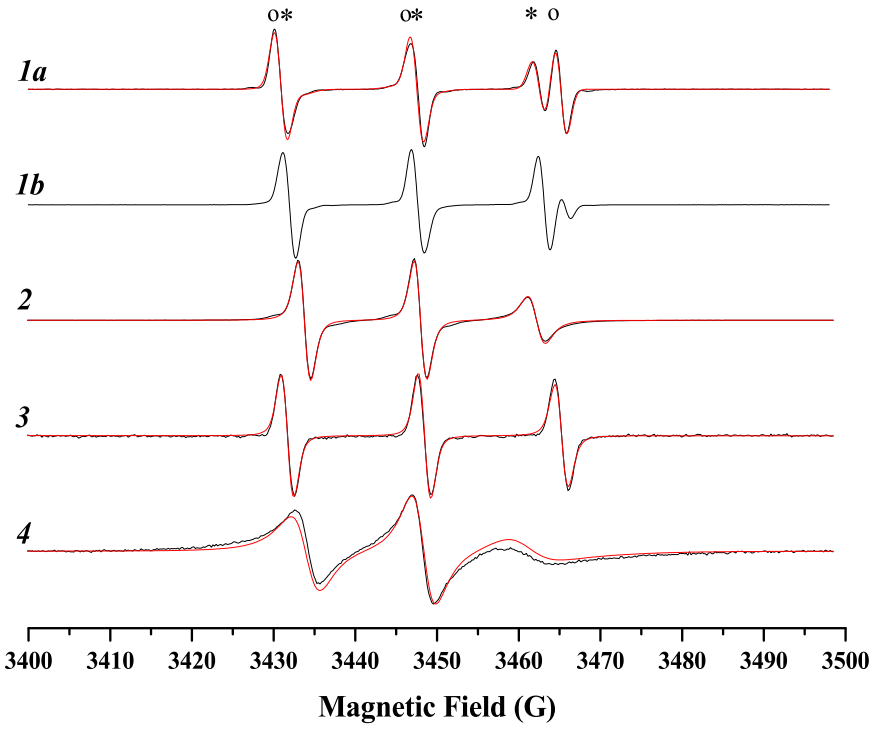


Fig. 3. The experimental (black line) and theoretical (red line) EPR spectra of spin probes measured in samples of goat milk and cream at temperature 293 K. The spin probes are 1a - TEMPO in goat milk, 1b - TEMPO in goat cream, 2 - 16-DSA in goat milk, 3 - CAT-1 in goat milk, and 4 - 5-DSA in goat milk. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
The correlation times and isotropic hyperfine constants of spin probes in the goat milk samples at temperature 293 K.

Spin probe	Correlation time, s	Hyperfine constant, G
TEMPO	9.1×10^{-11} (*)	17.2 (*)
	1.3×10^{-10} (o)	15.8 (o)
16-DSA	5.3×10^{-10}	14.3
CAT-1	5×10^{-11}	16.7
5-DSA	1.3×10^{-9}	14.8

medium in the cream of goat milk as compared to the milk itself. As reference, the water content in the cream is 66 ± 1.8% and 86.9 ± 1.4% in milk. The calculated correlation times imply free rotation of radicals in these regions.

It is known that the 16-DSA radical is poorly soluble in water and prefers to be localized in hydrophobic regions of heterogeneous systems [13,34–36], in our case, possibly inside casein submicelles near their hydrocarbon core or in the glyceride core of milk fat globules. Spectrum 2 in Fig. 3 shows the EPR spectrum of this radical in goat milk. The best coincidence of simulated spectrum with experimental one is observed for the correlation time of the probe rotation and the isotropic hcc constant presented in Table 1. Note that polarity of the hydrophobic region of localization of 16-DSA is lower than in "free" ionogenic micelles (the hcc constant of this radical in micelles of sodium dodecyl sulfate, dodecyltrimethylammonium, tetradecyltrimethylammonium, cetyltrimethylammonium bromides is in the range of 14.9–15.2 G) and non-ionic micelles based on dodecyl-substituted polyethylene glycol (a^N = 14.9 G) [12,32]. Comparing the spectrum of 16-DSA in milk sample with spectrum of this probe in ionic and non-ionic micelles [12,13,32], it can be noted that frequencies of molecular motions in these regions are close in value which indicates the similarity of the molecular organization of hydrophobic regions. We also note the isotropy of the probe rotation in this region of localization. Apparently, the hydrocarbon molecular chains that form the hydrophobic regions create a medium whose properties are very close to isotropic nature.

The cationic radical CAT-1 is hydrophilic and dissolves only in the polar regions of heterogeneous systems; it can also interact electrostatically with a negatively charged surface providing information on its structure and molecular dynamics [13,35]. Spectrum 3 in Fig. 3 shows the spectrum of this radical in goat milk. The correlation time and isotropic hcc constant (Table 1) of the radical calculated from the experimental spectra are close to the values characteristic of a "pure" aqueous solution of CAT-1 (in water $\tau = 6 \times 10^{-11}$ s and $a^N = 16.7$ G [35,37]), which indicates the absence of large ionic clusters in the hydrophilic regions of casein micelles and in the aqueous phase of milk. Apparently, charged phosphate groups are screened by hydrophobic regions of casein micelles which is consistent with the results of [21].

The 5-DSA spin probe is incorporated into the micellar phase in such a way that carboxyl group is located near the core–shell interface, while the paramagnetic fragment of probe is localized deeper in hydrocarbon core, at a distance 5–7 Å from core-shell interface [12,13,36,37]. The EPR spectrum of this radical is shown as Spectrum 4 in Fig. 3. The values of the correlation time of the probe rotation and the isotropic hcc constant of the 5-DSA radical in goat milk are presented in Table 1. Note that these values are close to their values in "free" ionic and non-ionic micelles [12,13,32]. Apparently, the molecular organization and polarity of the hydrocarbon layer near the interface is the same in all these hydrophobic regions.

It is also important to note that ultrasonic treatment of goat milk solutions at temperature of 296 K for 30 min does not affect the EPR spectra of all used radicals. This implies that ultrasound irradiation of such condition does not affect the molecular dynamics and organization of casein micelles and fat globules. At the same time as it is well known that ultrasonic treatment in various solutions leads to the generation of short-living radicals in them [38]. The formation of such radicals could also occur upon ultrasonic treatment of goat milk samples. To test this assumption, the 5,5-Dimethyl-1-pyrrolineN-oxide (DMPO) spin trap was used.

There was no spin adducts detected in goat milk solutions with

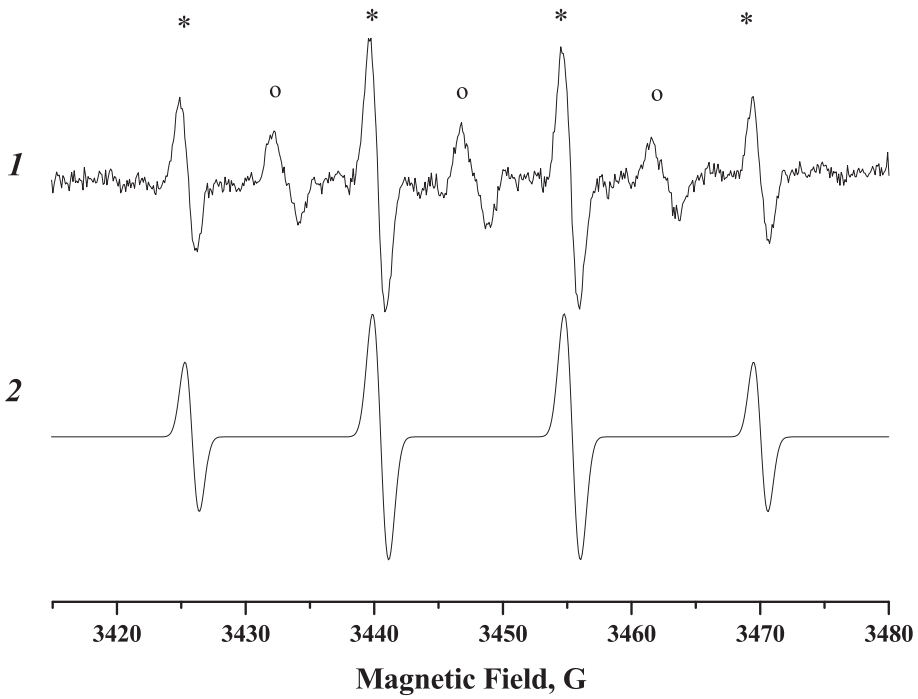


Fig. 4. Experimental (1) and simulated (2) spectra of DMPO spin trap after 30 min of ultrasonic treatment of aqua solution at temperature 296 K.

DMPO spin trap under ultrasonic treatment for 30 min. At the same time, the EPR spectrum was recorded under ultrasonic treatment of "pure" aqueous solution of the DMPO trap for 30 min at temperature of 296 K. Fig. 4 shows the EPR spectrum of the DMPO spin adducts. This spectrum consists of two EPR signals: a doublet of triplets (marked with * in Fig. 4) and a triplet (marked with o in Fig. 4). A triplet with hcc constant of $a^N = 14.7$ G refers to the decomposition products of the DMPO trap itself in solution [42]. The hcc constants of the doublet of triplets nitroxyl radicals calculated by simulation (Spectrum 2 in Fig. 4) - $a^N = 14.9$ Гс and $a^H = 14.7$ Гс – coincide with literature data for spin adducts of DMPO with OH radical [18,42]. The concentration of the OH-radicals in the aqueous solution after 30 min of ultrasonic treatment was $\sim 8.5\times10^{-7}$ mol/L. The absence of OH-radicals captured by the spin trap in the treated goat milk solutions is due to the "quenching" of short-living radical states by protein molecules of casein.

Note also that the localization regions of the spin probes are not precisely determined and is the subject of further research. However, in our opinion, spin probes reflect molecular mobility and microlevel organization of casein submicelles and protein globules. The invariability of their EPR parameters under ultrasonic treatment indicates that homogenization of goat milk occurs at the supra-macromolecular level and does not affect the microlevel.

3.2. NMR relaxation

The interaction of water with proteins, the fatty component of milk and lactose would most likely determine valuable qualities such as storage stability, taste, rheological properties, dehydration, and denaturation of proteins. When evaluating the molecular mobility by the proton magnetic relaxation times, one should consider the distribution of these values for water and fat. It was shown before [3,8] that the spin–spin relaxation time of fat, T_2 lies in the region of < 100 μs and greater than 100 μs for water, at a frequency of ~ 20 MHz; and these regions overlap. The obtained data of relaxation times for milk and cream of goat milk and anhydrous fat in the presence and absence (control) of ultrasound on milk are presented in Table 2. The decay of magnetization for milk sample was described by one exponent, and the decay curves for cream and fat of goat's milk were characterized by the sum of at least two exponents. Changes of this kind can be used to characterize change in the condition of fast exchange, when the diffusion times at distances comparable to the root-mean-square displacement become comparable to the relaxation times, or much longer than them. In this case, we observe relaxation in each separate phases of the protons.

Referring to the data in the table above, both the spin–lattice T_1 and spin–spin T_2 relaxation times for both the conditions in the absence and presence of ultrasound do not differ significantly. However, a different situation was observed with cream sample. There is a significant ($P < 0.05$) increase in water and fat protons relaxation times in the experiment under ultrasound treatment as compared to the control. Changes of this kind can also be reflected by changes in the pH, chemical exchange rates, redistribution of protons between phases, and changes in the

Table 2
The spin–lattice (T_1), the spin–spin (T_2) relaxation times and the diffusion coefficient D_{eff} in the goat milk samples with and without ultrasonic treatment. (The D_{eff} measurement was carried out at $\Delta = 7.5$ ms, $\delta = 500$ μs, $g = 1.74$ Tm^{-1}, n-number of samples, mean ± SE, % - impact of component).

	MILK		CREAM		FAT	
	Control	US treatment	Control	US treatment	Control	US treatment
T_1, s	1,47 ± 0,03 (n = 5)	1,48 ± 0,08 (n = 5)	1,32–74% 0,14–26%	1,49–81% 0,15–19%	0,33–47% 0,09–53%	0,38–40% 0,11–60%
T_2, ms	146 ± 15 (n = 5)	156 ± 18 (n = 5)	315–87% 44,3–13%	415–95% 67–5%	367–50% 73,7–50%	410–45% 80–55%
$D_{eff}*10^9$ m^2s^{-1}	1,77 ± 0,17 (n = 4)	2,21 ± 0,19 (n = 4)	1,36 ± 0,07 (n = 5)	1,54 ± 0,08 (n = 5)	0,043 ± 0,021 (n = 4)	0,068 ± 0,03 (n = 4)

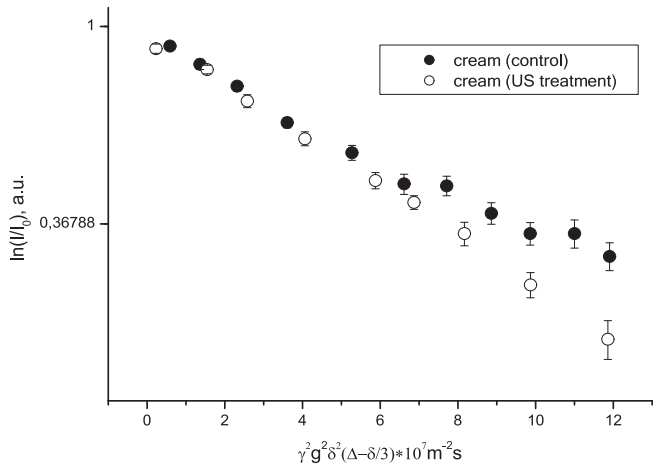


Fig. 5. Dependency of the relative spin echo signal with pulsed gradient (I) and without pulsed gradiaent (I_0) for cream samples under the influence of ultrasound (○) and control milk (●) on the value of the pulse gradient g. The diffusion time and pulse gradient duration are Δ = 7.5 ms and δ = 500 μs, respectively.

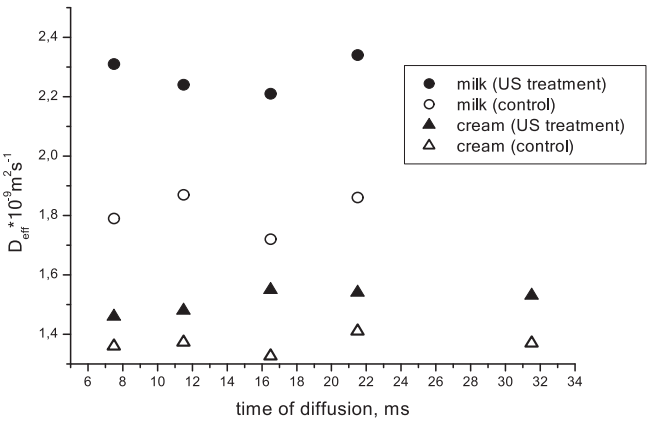


Fig. 6. The self-diffusion coefficient, D_{eff} in goat milk and cream samples in the presence and absence of ultrasound treatment. Dependence on the time of diffusion. The value and duration of the impulse gradient for a given measurement g = 1.74 Tm^{-1} and δ = 500 μs, respectively.

structure of the sample such as its porosity [40,41]. The authors of [3] note a change in the distribution of the T_2 relaxation time in milk cream, depending on the content of long-chain fatty acids. This may be a direct consequence of the action of ultrasound.

3.3. Diffusion

When studying molecular diffusion in milk, the presence of several proton-containing phases should be noted. Water, fatty acids, triglycerides and proteins that form globular and micellar formations add complexity to the assessment of the results obtained. The spin echo signal used to determine D is the sum of the signals of all protons with a specific D for each proton phase. Diffusion processes include diffusion of water protons, limited diffusion within fat globules and micelles, and diffusion of globules and micelles as a whole. The two-phase diffusion model [30], which is often used in this case, assumes that both phases' protons of the system make a contribute to the decay of magnetization with their own diffusion coefficients

Fig. 5 shows the dependence of the relative attenuation logarithm of the spin echo amplitude on the value $k = \gamma^2 g^2 \delta^2(\Delta\text{-}\delta/3)$ with a change in the value of the pulse gradient g for goat milk cream samples in the presence and absence of ultrasound. The amplitude decay for the samples under ultrasound treatment is approximated by a linear dependence (coefficient of determination $R^2 = 0.997$), which indicates that the diffusion is of a one component nature. This is confirmed by insignificant changes of D_{eff} in the cream samples with a change in the diffusion time, Δ (Fig. 6) and indicates the unlimited diffusion at a distance $(r^2) = 6 D_{eff}\Delta$ (~16μ). The decrease in D_{eff} as compared to D for pure water is determined by the change in viscosity due to the presence of casein micelles, fat globules, dissociated proteins and minerals.

As can be seen, the decay of the spin echo amplitude in the control cream samples is nonlinear. Changes of this kind indicate a multiphase distribution of the diffusion coefficients. If we assume that diffusion in milk is determined by the diffusion coefficient of water D_{H2O} and the average diffusion coefficient of non-aqueous protons D_{nw}, then Eq. (1) can be written as

$$A_n/A_0 = A_1/A_0 exp(-kD_{H_2O}) + A_2/A_0 exp(-kD_{nw}) \quad (2)$$

A_1/A_0 and A_2/A_0 are the relative contributions to the change in the spin echo signal under the action of a magnetic field gradient from aqueous and non-aqueous protons, respectively ($A_1/A_0 + A_2/A_0 = 1$), $k = \gamma^2 g^2 \delta^2(\Delta\text{-}\delta/3)$. Since the apparatus difficulties, due to the so-called "dead time", does not allow us to determine the intensities of signals from protons of water and non-aqueous protons at the beginning of the decay (t = 0), we determined the relative values of A_1/A_0 and A_2/A_0 from the approximation of the signal intensity dependence for samples with different moisture content (water content in goat milk cream = 66 ± 1.8%). The obtained values ($A_1/A_0 = 0.27 \pm 0.04$, $A_2/A_0 = 0.73 \pm 0.05$) are substituted into Eq. (2) together with the diffusion coefficient for pure water ($D_{H2O} = 2{,}3 \times 10^{-9}$ m^2 s^{-1}) allowed obtain the calculated value of $D_{nw} = (0.056 \pm 0.048) * 10^{-9}$ m^2 s^{-1}. D_{nw} describes the diffusion of both the fat globules and micelles themselves and of protons in them. The D_{fat} of anhydrous goat's milk fat (AMF) measured in our experiment give values $(0.043 \pm 0.021) \times 10^{-9}$ m^2 s^{-1}, which is similar to data for AMF obtained in (26) ($D_{fat} \sim 0.025 \times 10^{-9}$ m^2 s^{-1}). As can be seen from the data we obtained and as noted by the authors of (26), the low accuracy of D_{nw} values is determined by a small number of experimental points and the use of a low-frequency spectrometer (therefore, a low signal-to-noise ratio). The used range of values of the impulse gradient (0.4–2.5 Tm^{-1}) contributed to a decrease in the signal of protons of dehydrated goat fat by<10%, which significantly reduces the measurement accuracy and requires higher strength of the gradient. It was shown in [31] that for individual casein molecules dissociated in solution ($D_{nw} \sim 0.04 \times 10^{-9}$ m^2 s^{-1}), and diffusion of casein micelles in solution as whole ($D_{nw} \sim 10^{-13}$ m^2 s^{-1}), are much lower than the limits of our measurements. Comparison of the magnitudes of the obtained values D_{nw} and D_{fat} for AMF, literature data for the diffusion of fat and micellar protons allow us to conclude that the two-phase model is applicable to the description of diffusion in samples of goat milk cream. However, the measurement accuracy requires a large number of experimental points. The change in the nature of diffusion observed in the samples treated with ultrasound can most likely be associated with the homogenizing effect of ultrasound, the dispersion of globular and micellar formations.

4. Conclusion

The obtained values of the molecular motion frequencies and the spin probes hcc constant in goat milk indicate a similar molecular organization of the hydrophobic regions of the components with micelles surfactants with a C12-C16 hydrocarbon chain length. The absence of ionic clusters in the aqueous phase of milk as in the hydrophilic regions of casein micelles shown indicates the screening of charged phosphate groups by hydrophobic regions of casein micelles. Ultrasonic treatment at this power did not cause noticeable changes in local mobility and molecular organization in the studied hydrophobic and hydrophilic regions of goat milk components. The free radicals formed during this

treatment are not captured using DMPO spin trap, apparently due to competitive reactions with triglycerides, proteins, and fatty acids in goat milk. Changes in the values of spin–lattice T_1 and spin–spin T_2 relaxation times and diffusion coefficients recorded by the NMR relaxation method and the magnetic field gradient in the samples of ultrasound-treated goat milk products are apparently due to the dispersion of globular and micellar supramolecular associations, which is the homogenizing action of ultrasound.

CRediT authorship contribution statement

Andrey Sergeev: Conceptualization, Investigation, Writing - original draft. **Mikhail Motyakin:** Conceptualization, Investigation, Writing - original draft. **Irina Barashkova:** Methodology, Investigation. **Victoria Zaborova:** Writing - review & editing. **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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