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# The influence of unconventional ultrasonic pasteurization on the characteristics of curds obtained from goat milk with the low cholesterol content

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ABSTRACT

This study aimed to evaluate the influence of different power-time ultrasound regimes of pasteurization on the physical, chemical, organoleptic properties, and lipid quality indices of goat curds characterized by a low cholesterol level. Cholesterol was eliminated by a percentage of 92.1 % by treating the raw goat milk with beta-cyclodextrin in the proportion of 0.6 %. Afterward, the goat milk was subjected to the following ultrasound regimes: 320 W for 1 (PA1), 3 (PA3), and 6 min (PA6) and 881 W for 1 (PP1), 3 (PP3), and 6 min (PP6) and then used for the curds production. Due to the ultrasound treatment, the milk suffered a concentration phenomenon, the most accentuated being registered for the PP6 sample. Considering the sensory properties, the most appreciated curd was the one obtained by the PP6 regime which recorded the highest scores for color and taste. Regarding the microbiological aspects, the ability of ultrasounds to inactivate microorganisms is observed and the most accentuated phenomenon is reported in the PP6 case. Thus, in comparison with the control sample, the total number of germs is reduced by a proportion of 91.85 %, the β-glucuronidase-positive *Escherichia coli* decreased by 93.15 %, while the coagulase-positive staphylococci were completely inactivated for the PP6 curd. The curds obtained for the PA6 and PP6 regimes registered the highest dry matter values as a cause of an accentuated syneresis process. The acidity values were higher for the curds obtained for PA1, PA3, and PA6 regimens due to more pronounced lactose hydrolysis and lower in the cases of PP3 and PP6 regimens compared to the control cheese. Twenty-five saturated, monounsaturated, and polyunsaturated fatty acids were identified in the curd samples and a rise in the unsaturated fatty acids proportion as the intensity of the applied ultrasound regime increased was observed. Also, AI, TI, and H/H lipid quality indices recorded better values as the power and time of the ultrasound action increased.

## 1. Introduction

Goat milk and its derived products were reported for their functional role in maintaining the nourishment and health of the young and elderly people [1], being especially an important source of calcium, protein, thiamine, niacin, and magnesium [2]. Compared to other types of milk, it has better digestibility, buffer capacity, alkalinity, and fat with better physical properties (surface tension, viscosity, and specific gravity) [3]. Considering the recommendations of the World Health Organization and the American Heart Association to decrease the consumption of cholesterol and saturated fats to reduce the risk of coronary heart disease, an improvement can be given to these already functional products by the action of reducing the cholesterol content [4]. Many methods for food cholesterol reduction have been studied including blending in vegetable oils, extraction by distillation and crystallization, adsorption with saponin and digitonin, and removal using supercritical carbon dioxide, but they all involve a series of disadvantages (organic solvents, residues, operation costs, etc.) [5,6]. Further research recommends the use of beta-cyclodextrin (β-CD) as being very efficient for cholesterol removal from dairy products [6]. β-CD is a non-toxic and non-digestible

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cyclic oligosaccharide with an affinity for non-polar molecules like cholesterol [4]. It has a very high capacity to reduce milk cholesterol, which is eliminated in proportions up to 97 % [4,6].

Very appreciated and found especially in the local markets, the Romanian goat curd is a semi-soft, fresh, most often unsalted white cheese made from raw milk, described as very refreshing with a slightly acidic flavor [7]. Because it is not usually obtained from pasteurized milk, the curd can generate several food hazards, especially of a microbiological nature. Thus, the unconventional pasteurization processes, which will not cause organoleptic and nutritional changes in the finished products are gaining more and more importance among food technologies. Considering these minimal processes applied to milk, the non-thermal ultrasound (US) emerging technology seems to be most suitable for pasteurizing goat milk used in cheese production. So, according to Zhao et al. [8] the US treatment of goat milk, which usually has a poor coagulation ability characterized by the formation of a less hard and more fragile gel with more protein particle loss due to more whey separation, improved its coagulation properties. In their study, gel firmness, coagulum strength, final storage modulus, cohesiveness, water holding capacity, and cross-linking of gels had a significant increase [8]. This enhancing evolution seems to be generated by many factors like the lower casein content (particularly the low proportion of $\alpha_{s1}$-casein), the higher degree of casein micelle dispersion, the differences in casein micelle composition, size and hydration, the mineral concentration and $\alpha_{s1}$-casein polymorphism [9,10,11]. Superior renneting properties generated by the US treatment were also observed by Liu et al. [12] and these were attributed to the physical effects of the cavitation phenomenon: milk particles size reduction and possible changes in protein hydrophobicity [12].

The ultrasound non-thermal technology is represented by the action of the high-frequency sound waves exerted over the human hearing threshold (≈ 20 kHz) and leads to alternating high and low pressures that are causing the rarefraction phenomenon characterized by compression and expansion cycles which finally generates the acoustic cavitation or implosion. Thus, during the negative pressure cycle, tiny vacuum bubbles (cavitation bubbles) occur and start to develop throughout several cycles till they reach the maximum amount of energy they can absorb. At this point, the cavitation bubbles suffer the implosion phenomenon and generate physical and chemical effects like microstreaming, agitation, turbulence, shock waves, highly reactive radicals' formation, etc. [13]. The micro implosions can provoke extreme conditions of temperatures and pressures responsible for very high shear forces formation [14]. Those ultrasound-induced effects became promising in the food products processing, preservation, and safety, especially in the dairy industry because they allow milk processing with reduced changes due to the mild thermal treatment [15]. So, the use of ultrasounds in dairy processing became an alternative to fat globules homogenization, lactose crystallization changes, microbial suppression, fermentation rates, and probiotic survival modification, enhancement of the bioactive activity, and transformations of the rheological properties [16–20]. The ultrasound treatment is also justified by shorter processing times, reduced water and energy costs, and lower generation of residual effluents and toxic compounds, besides preserving the nutritional values and the sensory characteristics of food [17,21].

To the best of our knowledge, there is no research on the effects of different power-time ultrasound regimes of pasteurization on the physical, chemical, organoleptic properties, and lipid quality indices of Romanian goat curds characterized by a low cholesterol level. Hence, the objective of this study was to investigate those effects by applying different methods of physical-chemical, microbiological, and sensorial analyses to the obtained goat curds.

## 2. Materials and methods

### 2.1. Materials

Raw goat milk was purchased from the local market in Baia Mare, Romania, transported in isothermal conditions, and refrigerated (≈ 4 °C) until later processing. High purity beta-cyclodextrin was purchased from Chengdu Healthlife Biotechnology Co., China, and all solvents and reagents were of analytical grade.

### 2.2. Beta-cyclodextrin treatment of goat milk for the cholesterol reduction

To reduce the cholesterol content of the goat milk, the slightly modified method of Alonso et al. [4] was applied. Beta-cyclodextrin was added to 7 L of milk in the proportion of 0.6 %, then they were mixed for 25 min and left static at 4 °C for 8 h. The mixture was passed through the BORALSAN centrifugal separator (Model 100–18, with a capacity of 100 L/h), at the base of which the beta-cyclodextrin-cholesterol residue was removed. Afterward, the goat milk was directed for unconventional pasteurization at different power-time ultrasound regimes.

### 2.3. Ultrasound (US) processing of milk

The goat milk was subjected to various ultrasonication regimes at the frequency of 40 kHz and the constant temperature of 20 °C using the Ultrasound bath HM1005.

To evaluate how the ultrasound treatment affects the goat milk used for curds production different conditions of power, time, and implicit energy density were applied. Thus, the samples subjected to ultrasounds non-thermal pasteurization are presented in Table 1.

After the ultrasound treatment, goat milk samples were collected for the physical-chemical assessment, and the rest of the milk was used to produce the 7 different goat curds.

### 2.4. Physical-chemical characteristics and cholesterol content assessment of the goat milk

The physical-chemical analysis of the different US pasteurized types of milk was performed using the LactoStar FTIR Milk Analyzer (Funke Gerber), which works based on a combined thermo-optical analyzing procedure.

Cholesterol extraction and determination were made according to the method applied by Dumuta et al. [22], thereby: 10 mL of raw or treated milk was saponified at 60° C for 30 min with 50 mL 2 M KOH alcoholic solution and cooled at room temperature; then the cholesterol was extracted with 50 mL hexane, evaporated until dryness and redissolved with 10 mL of 2 propanol, passed through the 0,2 μm Chroma fill syringe filters and injected into the chromatograph YL9100 HPLC with UV-vis detector at 212 nm. This is characterized by the following components: vacuum degassing system of the solvent, quaternary pump for 4 solvents, Eclipse XDB-C18 column filled with silica gel with chemically grafted octadecyl groups, of the dimensions 250 x4.6 mm (length × diameter), with pores of 5 μm and operated in the conditions: isocratic elution using a mobile composition phase of 75 % methanol and 25 % isopropanol HPLC purity, a flow rate of 1 mL/minute, the

**Table 1**
Various regimes of the ultrasound treatment of the goat milk.

| Sample | Power, W | Time, minutes | Energy density (J/ml) |
|---|---|---|---|
| C, control sample | – | – | – |
| PA1 | 320 | 1 | 1.28 |
| PA3 | 320 | 3 | 3.84 |
| PA6 | 320 | 6 | 7.68 |
| PP1 | 881 | 1 | 1.762 |
| PP3 | 881 | 3 | 5.286 |
| PP6 | 881 | 6 | 10.572 |

temperature in the thermostatic column compartment of 30° C and the manual injection using a Hamilton syringe.

### 2.5. Curds production

The US pasteurized milk used for obtaining this type of cheese is heated to 30–31° C for the rennet addition; then the milk is left at the same temperature for coagulation over 30–45 min. The coagulated mass is cut into cubes and passed in gauze cloths for whey separation and maturation. All 7 types of curds are subjected to ripening operation at a temperature of 12–16° C for 3 days [23]. Samples will be taken and frozen for further analysis.

### 2.6. Sensory evaluation of the goat curds

To evaluate the sensory aspects of the goat curds, ten trained panelists were selected to examine their exterior appearance, color, taste, smell, consistency, and section aspect on a scale from 0 to 5.

### 2.7. Microbiological assessment of the curds

The microbiological assessment of the curds consisted of analyses considering: the total number of germs [24], the number of *Escherichia coli* colony-forming units positive for beta-glucuronidase [25], and the number of staphylococci coagulase positive [26].

To enumerate the viable bacteria, present in the milk samples, serial dilutions of the samples in sterile saline in a range of $10^{-1}$ to $10^{-7}$ were conducted, followed by dispersion of 1 mL of each dilution on agar media. PCA medium was used to highlight the total number of germs, TBX medium was used to count the E. coli positive for beta-glucuronidase, and Baird-Parker egg yolk tellurite agar medium was used to determine the number of staphylococci coagulase positive. After incubation, the colonies were counted and the number of colony forming units (CFU)/g of goat curd was calculated. To meet the criteria of statistical accuracy for the bacteria number of the given samples, three replications are made and calculated as the total number of colonies forming units only from the plates showing between 20 and 200 visible colonies.

### 2.8. Physical-chemical analyses of the goat curds

The different types of curd were analyzed considering the: dry matter, % (w/w) and water content, % (w/w) by drying in the oven at $102 \pm 2$ °C, and acidity, T degree (mEq acid/100 g) using the Thörner method [27].

### 2.9. Analysis of the fatty acids profile of curds by GC

The fatty acid methyl ester analysis of the curds was performed using an Agilent 7890 gas chromatograph with a flame ionization detector. Samples with known amounts were placed in a 50 mL Erlenmeyer flask with 20 mL of isooctane and 2.5 mL methanolic potassium hydroxide, 2 molL$^{-1}$, and allowed to extract in an ultrasonic water bath at 80 °C for 20 min. The filtered solvent solution was neutralized by 1 g of sodium hydrogen sulfate monohydrate.

1 mL of the upper phase was transferred into a 2 mL vial and 1 μL of each sample was introduced into the gas chromatograph equipped with a DB-Wax fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The instrument conditions were as follows: the injector temperature was set at 250 °C with split less mode; helium was used as carrier gas with a flow rate of 1 mL·min$^{-1}$; column temperatures from 100 to 180 °C at a rate of 7 °C min$^{-1}$ with 5 min of isothermal regime followed by 180–240 °C at a rate of 10 °C min$^{-1}$ with another 10 min of isothermal time. The fatty acid methyl esters were identified by comparing the retention times with those of standard compounds which were then compared with the retention time of the methyl esters of fatty acids of reference milk fat. The results of the analysis are expressed as a weight percentage of individual fatty acid in relation to the sum of all fatty acids (FAs) detected.

### 2.10. Lipid quality indices

Considering the fatty acids composition, lipid quality indices such as Index of atherogenicity, AI, Index of Thrombogenicity, TI, and hypocholesterolemic/hypercholesterolemic ratio, H/H were calculated based on the formulas proposed in the literature [28,29]. Thus, the influence of different ultrasound pasteurization regimes on the nutritional indices of the curds was assessed.

Index of Atherogenicity (AI) was calculated based on the Eq. (1).

$$AI = (C12:0 + (4 \times C14:0) + C16:0) / (\Sigma n-3\ PUFA + \Sigma n-6\ PUFA + \Sigma\ MUFA) \quad (1)$$

AI indicates the relation between the sum of main saturated fatty acids and the sum of unsaturated fatty acids. C12:0, C14:0, and C16:0 favor the adhesion of lipids to cells of the circulatory and immunological system while the unsaturated fatty acids are anti-atherogenic due to their effect on inhibition of the plaque accumulation and reduction of phospholipids, cholesterol, and esterified fatty acids levels [29,30].

Index of Thrombogenicity (TI) was calculated according to Eq. (2):

$$TI \frac{C14:0 + C16:0 + C18:0}{(0.5 \times \sum MUFA) + (0.5 \times \sum (n-6) PUFA) + (3x \sum (n-3) PUFA) + (n-3)/(n-6)} \quad (2)$$

The TI characterizes the thrombogenic potential of FAs, indicating the tendency to form clots in blood vessels and provides the contribution of different FAs, which indicates the relationship between the fatty acids (C12:0, C14:0, and C16:0) that favor the thrombus formation and the monounsaturated fatty acids and n-3 and n-6 polyunsaturated fatty acids considered anti-thrombogenic substances. Related to the TI index, the consumption of foods with a lower TI is beneficial for cardiovascular health [29,31].

The ratio of hypocholesterolemic and hypercholesterolemic fatty acids (H/H) was calculated according to the FAs composition using the equation [3]:

$$H/H = (C18:1n-9 + C18:2n-6 + C18:3n-3)/(C12:0 + C14:0 + C16:0) \quad (3)$$

The H/H ratio is connected to the functional activity of the fatty acids in the lipoprotein's metabolism for plasma cholesterol transport and to the risk of cardiovascular disease development. Higher values of this ratio are helpful considering cardiovascular disease prevention [28].

**Table 2**
Values of the physical-chemical characteristics of goat milk subjected to different ultrasound pasteurization regimes (average values of three samples and standard deviation).

| Sample | Fat % (w/w) | Proteins % (w/w) | Lactose % (w/w) | Non-fat dry matter (w/w) | Freezing point, °C | Minerals % (w/w) |
|---|---|---|---|---|---|---|
| C, control sample | 3.18 ± 0.010 a * | 3.24 ± 0.01 a | 4.32 ± 0.026 a | 12.13 ± 0.026 a | −0.535 ± 0.001a | 0.97 ± 0.01a |
| PA1 | 3.26 ± 0.020b | 3.31 ± 0.02b | 4.43 ± 0.02b | 12.40 ± 0.020b | −0.541 ± 0.001b | 0.97 ± 0.01a |
| PA3 | 3.31 ± 0.029c | 3.34 ± 0.02c | 4.47 ± 0.02c | 12.51 ± 0.010 d | −0.546 ± 0.001d | 0.97 ± 0.01a |
| PA6 | 3.28 ± 0.013bc | 3.34 ± 0.02 bc | 4.47 ± 0.03c | 12.52 ± 0.017 d | −0.546 ± 0.001 d | 0.97 ± 0.01a |
| PP1 | 3.27 ± 0.021bc | 3.34 ± 0.02 bc | 4.45 ± 0.01 bc | 12.45 ± 0.017c | −0.543 ± 0.001c | 0.97 ± 0.01a |
| PP3 | 3.28 ± 0.026bc | 3.32 ± 0.017 bc | 4.47 ± 0.01c | 12.52 ± 0.010d | −0.545 ± 0.001 d | 0.98 ± 0.01a |
| PP6 | 3.80 ± 0.037 d | 3.34 ± 0.026 d | 4.47 ± 0.02c | 12.53 ± 0.017d | −0.545 ± 0.001 d | 0.98 ± 0.01a |

* Different letters in the same column indicate statistically significant differences at P = 95 % according to Fisher's least significant difference (LSD) procedure.

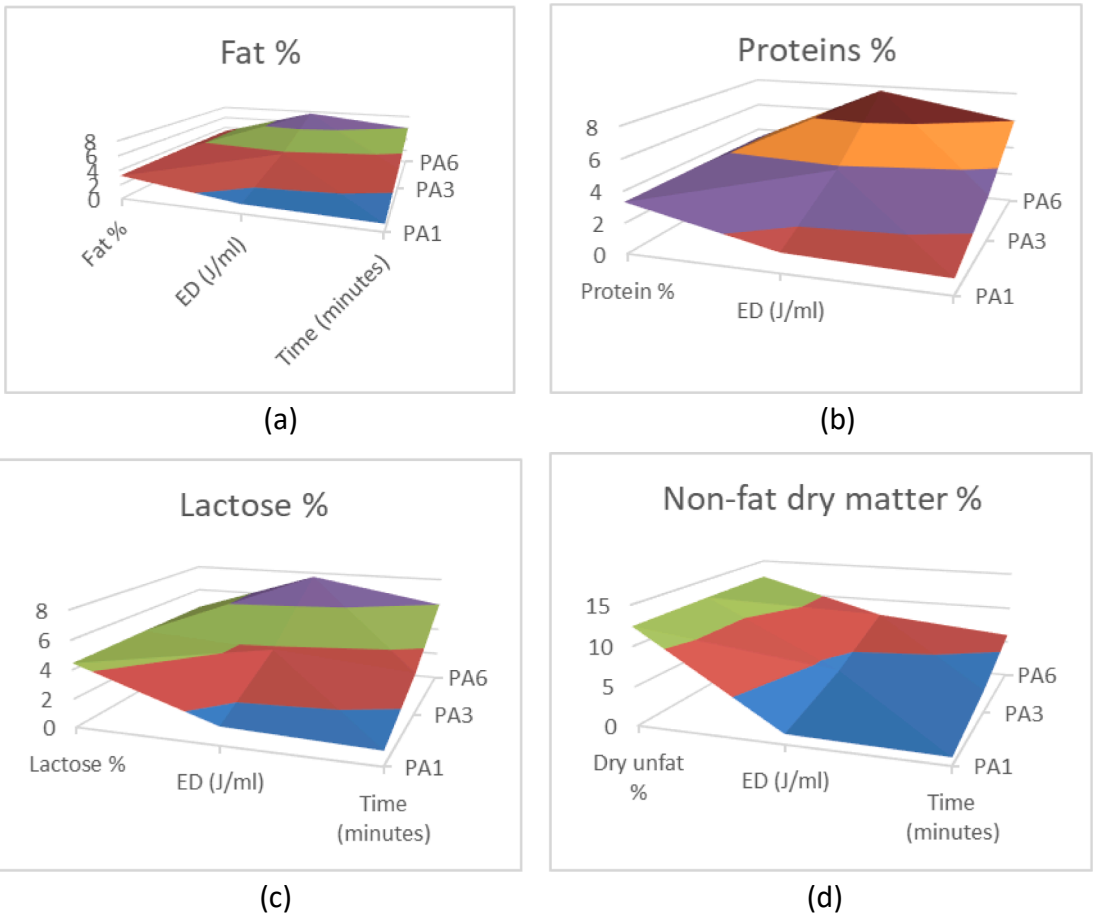


**Fig. 1.** Fat (a), proteins (b), lactose (c) and non-fat dry matter (d) content values of the 320 W ultrasound power treated milk.

## 3. Results and discussion

### *3.1. Physical-chemical characteristics and cholesterol content of the US-treated goat milk*

#### *3.1.1. Physical-chemical characteristics*

The results of the physical-chemical analysis of the US-treated goat milk are presented in Table 2.

The variations of the goat milk composition content considering different power-time ultrasound regimes are represented in the following graphics (Figs. 1 and 2).

The ultrasonic treatment of various food products is known to bring several advantages in terms of chemical composition. The physical-chemical parameters of dairy products have a very important role in their quality assessment [13].

In this study, the main observed phenomenon is the concentration of the milk samples caused by water evaporation. Thus, at the highest used power and for the longest exposure time, the most accentuated concentration is observed. In this sense, the fastest and most accentuated increase of the values for the non-fat dry matter, and for the fat content can be observed in the case of the treatment applied at 881 W for 6 min (sample PP6). This effect is also confirmed by the literature, which shows an increase in the total dry matter and a decrease in the milk pH without affecting its color significantly in the ultrasound application case [20,32].

Due to the ultrasonication processing, a change in the size of the milk components is reported with favorable effects on the finished products [13]. Thus, the fat globules membrane is affected, which determines the reduction of the fat globules' size and a granular surface of the globules due to the interaction of casein micelles with the affected membrane [33]. This phenomenon of casein adsorption on the surface of fat cell membranes has also been observed by Karlović et al. [34] in ultrasound-

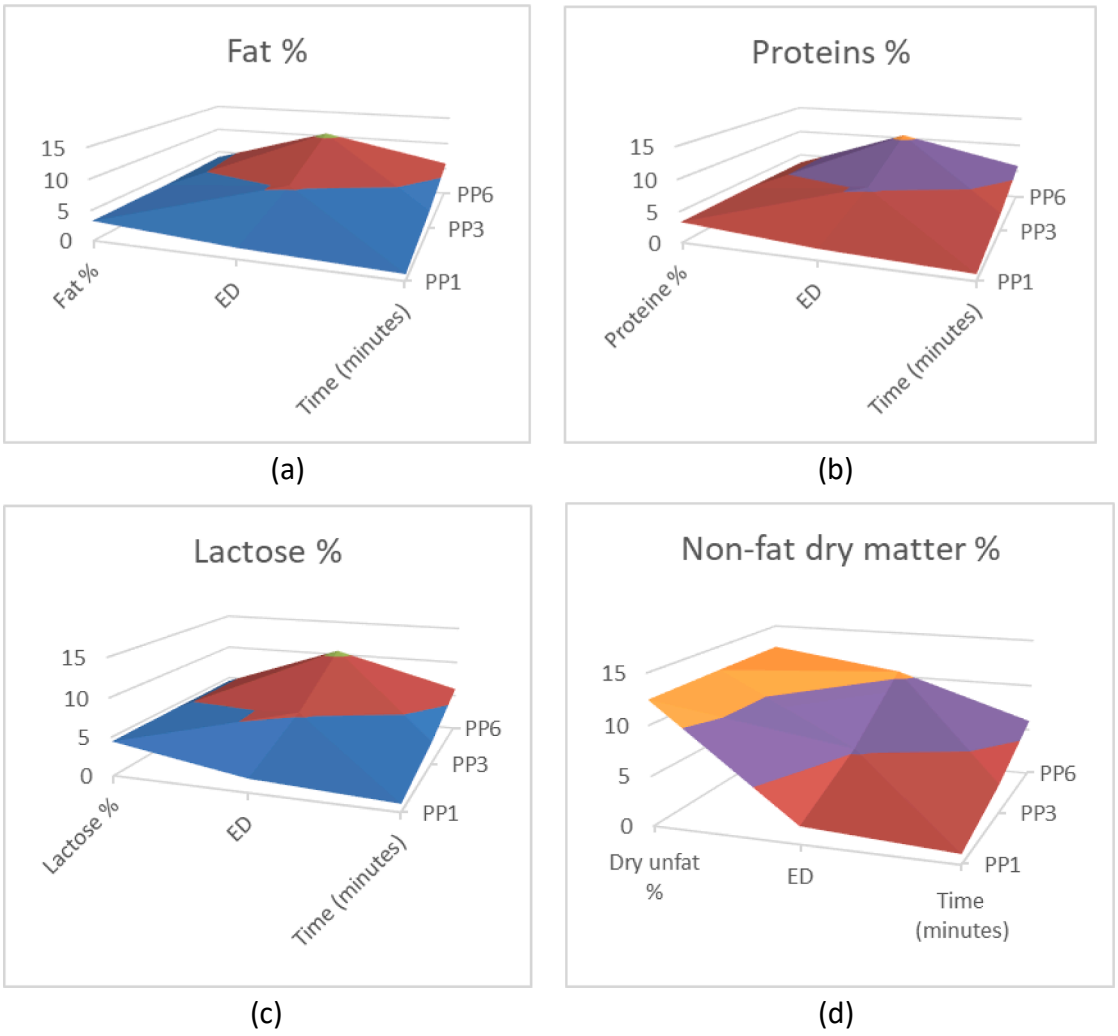


**Fig. 2.** Fat (a), proteins (b), lactose (c) and non-fat dry matter (d) content values of the 881 W ultrasound power treated milk.

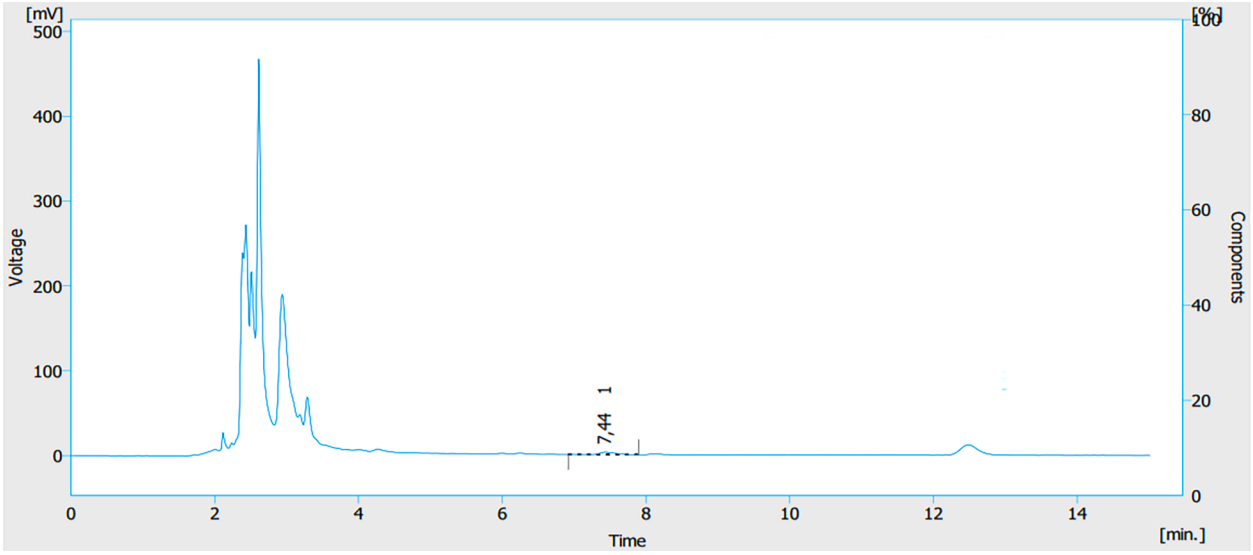


**Fig. 3.** Chromatogram for the cholesterol content determination of the milk treated with beta-cyclodextrin.

treated sheep milk samples and they considered that caseins act as natural emulsifiers.

Also, the serum proteins are denatured and form soluble compounds such as serum-serum/serum-casein [35]. Wu et al. [36] conclude that the action of ultrasound affects serum proteins by enhancing enzymolysis which leads to the formation of functional compounds represented by newly formed bioactive peptides.

The milk freezing process is also influenced by the ultrasound treatment. Thus, a reduction in the size of the ice crystals may take place, leading to a decrease in the freezing time [37]. This can be confirmed by the values obtained for the freezing point, which records the fastest decrease in the case of US treatment at the highest power (PP1 sample).

#### 3.1.2. Cholesterol content of beta-cyclodextrin-treated goat milk

The chromatogram of the goat milk treated with beta-cyclodextrin to reduce the cholesterol content is presented in Fig. 3.

From the previous figure, for the low-cholesterol goat milk sample, the cholesterol peak appeared at 7.44 min. Also, the program of the device indicated an area of 47.057 mV × s, and thus, considering the cholesterol standards too, the cholesterol content of the treated milk was determined equivalent to 0.87 mg %. Taking into account that the average goat milk cholesterol content is 11 mg %, a cholesterol content reduction of about 92,1 %, can be calculated. This amount is in accordance with the values found in the specialty literature [4,5,38,39].

The lowering of cholesterol content by beta-cyclodextrin treatment of milk contributes to the obtaining of healthier dairy products with better nutritional values. Christoforides et al. [40] explain the cholesterol reduction by the attachment to beta-cyclodextrin via its axial encapsulation in a head-to-head β-cyclodextrin dimer and numerous van der Waals and C—H···O bounds to the inner host cavity.

### 3.2. Sensory properties of the goat curds

Ultrasonication influenced the organoleptic properties of the cheeses obtained from the goat milk subjected to different unconventional pasteurization regimes. Sensory scores were higher for the curds obtained from the milk treated for a longer period because of the proteolysis and lipolysis phenomena. The color and taste of the PP6 curd were the most appreciated, especially compared to the control cheese. The study results agree with the observations reported by Bermudez-Aguirre et al. [33] and Jalilzadeh et al. [41]. Also, the smell and consistency scores were higher in the case of the cheeses obtained from the goat milk subjected for a longer period to the ultrasound action, while the exterior appearance and section aspect was less appreciated due to the more traditional way of forming and ripening of the curds. The most appreciated was the curd obtained from the milk treated at ultrasounds for 6 min at a power of 881 W (PP6) (Fig. 4).

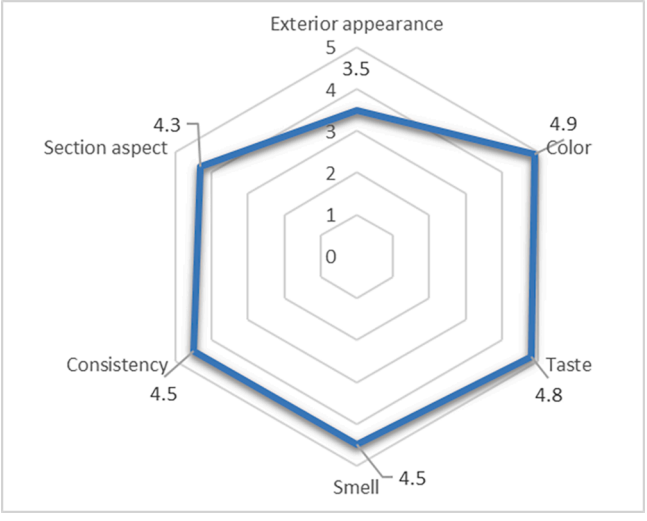


**Fig. 4.** Sensory assessment of the curd obtained from the milk treated at ultrasounds for 6 min at a power of 881 W (PP6).

### 3.3. Microbiological assay of the curds

#### 3.3.1. The total number of germs

Fig. 5 shows the antimicrobial action of the ultrasound treatment influenced by the applied power. Regarding the application of the lower power treatment (320 W), the most marked decrease was observed in the case of the sample subjected to the longest time interval (PA6) achieving a reduction of 89.92 % of the total number of germs. The application of the higher power treatment (881 W) showed the same type of variation, also with a higher reduction of the total number of germs, about 91.85 %.

These reduction rates show the ability of unconventional ultrasound technology to inactivate the microorganisms. Thus, the microbubbles formed by the acoustic cavity generated by this technique collapse and lead to the appearance of micro shear rates that damage the bacterial wall [42].

The number of mesophilic bacteria decreased significantly during these different power-time ultrasound regimes. The results obtained by Herceg et al. [43] also indicate a significant inactivation of microorganisms over longer periods of ultrasound treatment, especially in combination with high temperature and amplitude. However, research in the field shows that ultrasound treatment is still controversial given that mesophilic bacteria in milk include both Gram-positive and Gram-negative, and their sensitivity is not sufficiently studied [44,45]. Gram-positive bacteria appear to be more resistant to various physical or chemical aggressions compared to Gram-negative ones because they have a thicker cell wall with higher concentrations of peptidoglycan [13].

#### 3.3.2. Number of Escherichia coli colony-forming units positive for beta-glucuronidase

In Fig. 6, the presence of *Escherichia coli* in the control sample was detected as being above the maximum limit allowed (1000 CFU/g). In the curds obtained from the US treated milk, a significant colonies reduction was observed, especially for the samples treated for the longest period, of about 6 min. When the power of 320 W was applied for 6 min, the reduction was 66.84 %, while the milk treatment at 881 W for the same time interval conducted to a diminution of 93.15 % of the *Escherichia coli.*

*Escherichia coli* contamination, which is an indicator of fecal nature, is common in milk and dairy products. Although E. coli is reported to be destroyed by pasteurization, some strains, including the pathogenic strain O157:H7, may survive and form biofilms in the pasteurization equipment. Thus, various methods of inactivation have been tried, and the obtained results show the ultrasound's ability to reduce pathogens in the cheese, which can greatly reduce the incidence of food poisoning. Jalilzadeh et al. 2018 [41] showed the inactivation of E. coli O157: H7 (gram-negative bacteria) at 20, 40, and 60 kHz from cheese samples, and Cameron et al. [46] indicated that the number of E. coli was reduced by 100 % after 10 min of ultrasound treatment [41,46].

#### 3.3.3. The number of staphylococci coagulase positive

In Fig. 7, the presence of staphylococci coagulase positive in the control sample is above the maximum allowed limit (in dairy products values must be below 1000 CFU). The obtained value indicates that in the control sample the number is exceeded about six times. In contrast, in the samples subjected to the ultrasound action, the number decreases progressively with power increasing, to even being absent when subjected for 3 min and 6 min at the power of 881 W.

Thus, coagulase-positive staphylococci were completely inactivated

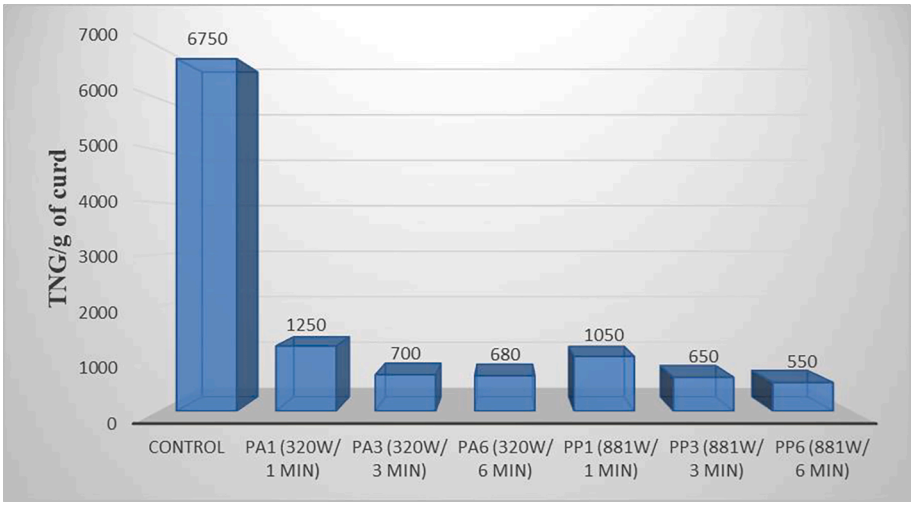


**Fig. 5.** Total number of germs values (TNG/g) for the 7 types of curds.

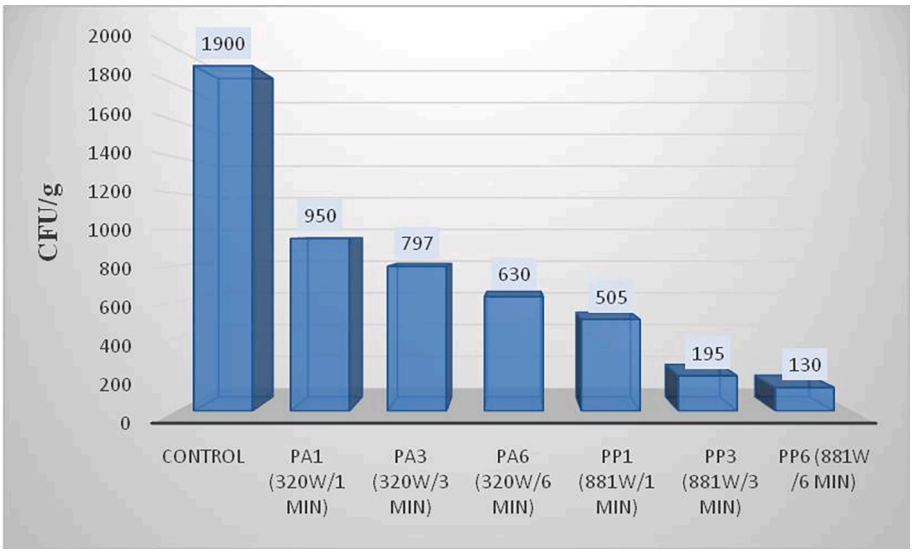


**Fig. 6.** Values of the β-glucuronidase-positive *Escherichia coli* colony-forming units for the 7 types of curds.

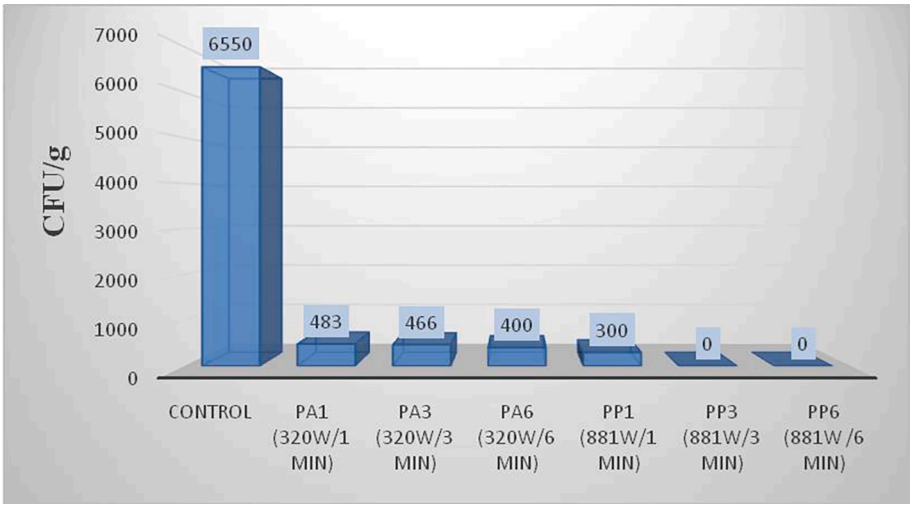


**Fig. 7.** Staphylococci CFU values of the 7 types of curds.

**Table 3**
Values of the physical–chemical characteristics of the curds obtained from the goat milk subjected to different power-time ultrasound regimes (average values of three independent samples and standard deviation).

| Sample | Dry matter, % (w/w) | Water, % (w/w) | Acidity, T degree (mEq acid/100 g) |
|---|---|---|---|
| C, control sample | 50.93 ± 0.99 a* | 49.00 ± 1.03 e | 293.24 ± 1.09c |
| PA1 | 51.5 ± 0.91 a | 48.35 ± 0.64 e | 326.75 ± 1.82f |
| PA3 | 54.3 ± 1.23 bc | 45.60 ± 0.96 cd | 320.75 ± 1.14 e |
| PA6 | 56.85 ± 0.81 d | 43.05 ± 0.71 a | 302.50 ± 1.45 d |
| PP1 | 55.33 ± 0.94 cd | 44.55 ± 0.64 bc | 295.10 ± 1.64c |
| PP3 | 53.3 ± 0.59b | 46.55 ± 0.80 d | 270.00 ± 1.31b |
| PP6 | 56.3 ± 0.48 d | 43.55 ± 0.66 ab | 250.15 ± 1.47 a |

* Different letters in the same column indicate statistically significant differences at P = 95 % according to Fisher's least significant difference (LSD) procedure.

in the cheese samples obtained from the goat milk subjected to higher power-time ultrasound regimes, which proves the effectiveness of the ultrasound action on pathogens. This was also highlighted by the research conducted by Jalilzadeh et al. [41] on Feta cheese in which the inactivation of *S. aureus* was found at 20 and 40 kHz.

### 3.4. Physical-chemical properties of the goat curds

The physical-chemical analysis results for the curds obtained by the coagulation of the 7 different types of milk are depicted in Table 3.

The higher dry matter values recorded for the cheeses obtained from the goat milk US treated for a longer period can be explained by an increased syneresis process, also observed in the literature [45].

The increased acidity values recorded for the curds obtained for PA1, PA3, and PA6 regimens are caused by more pronounced lactose hydrolysis. This may be due to the positive ultrasound action on the beneficial strains present in milk, mainly to the breakdown for the release of extra and intracellular hydrolytic enzymes, as well as to changes in the number of viable cells [47,48]. Also, the cheeses obtained

**Table 4**
Lipids fatty acids composition of the curds obtained from goat milk subjected to various power-time ultrasound regimes.

| Fatty acid in curd, % of total methyl esters* | Control | PA1 | PA3 | PA6 | PP1 | PP3 | PP6 |
|---|---|---|---|---|---|---|---|
| Butyric acid C4:0 | 1.95 ± 0.04$^{d}$ ** | 1.40 ± 0.05$^{b}$ | 2.14 ± 0.03$^{e}$ | 2.11 ± 0.05$^{e}$ | 1.58 ± 0.02$^{c}$ | 1.26 ± 0.01$^{a}$ | 1.60 ± 0.05$^{c}$ |
| *n*-caproic acid C6:0 | 1.96 ± 0.05$^{c}$ | 1.88 ± 0.06$^{c}$ | 2.18 ± 0.06$^{d}$ | 2.12 ± 0.04$^{d}$ | 1.59 ± 0.05$^{b}$ | 1.25 ± 0.04$^{a}$ | 1.59 ± 0.08$^{b}$ |
| Caprylic acid C8:0 | 2.01 ± 0.04$^{c}$ | 1.95 ± 0.05$^{c}$ | 2.29 ± 0.03$^{e}$ | 2.22 ± 0.03$^{d}$ | 1.62 ± 0.03$^{b}$ | 1.24 ± 0.05$^{a}$ | 1.62 ± 0.02$^{b}$ |
| Capric acid C10:0 | 5.56 ± 0.14$^{c}$ | 5.53 ± 0.11$^{c}$ | 6.56 ± 0.09$^{d}$ | 6.46 ± 0.11$^{d}$ | 4.60 ± 0.12$^{b}$ | 3.37 ± 0.07$^{a}$ | 4.65 ± 0.08$^{b}$ |
| Hendecanoic acid C11:0 | 0.06 ± 0.01 $^{cd}$ | 0.04 ± 0.01$^{ab}$ | 0.07 ± 0.01$^{d}$ | 0.07 ± 0.01$^{d}$ | 0.05 ± 0.01$^{bc}$ | 0.03 ± 0.01$^{a}$ | 0.05 ± 0.01$^{bc}$ |
| Lauric acid C12:0 | 1.93 ± 0.07$^{b}$ | 1.94 ± 0.05$^{b}$ | 2.41 ± 0.06$^{c}$ | 2.49 ± 0.08$^{c}$ | 1.68 ± 0.09$^{a}$ | 1.65 ± 0.11$^{a}$ | 1.62 ± 0.06$^{a}$ |
| Tridecylic acid C13:0 | 2.22 ± 0.08$^{d}$ | 1.53 ± 0.09$^{bc}$ | 1.42 ± 0.07$^{ab}$ | 1.36 ± 0.07$^{a}$ | 1.58 ± 0.08$^{c}$ | 1.41 ± 0.11$^{ab}$ | 1.31 ± 0.07$^{a}$ |
| Myristic acid C14:0 | 7.67 ± 0.16$^{d}$ | 7.87 ± 0.18$^{d}$ | 6.49 ± 0.14$^{bc}$ | 6.70 ± 0.19$^{c}$ | 6.39 ± 0.14$^{b}$ | 5.85 ± 0.12$^{a}$ | 5.62 ± 0.09$^{a}$ |
| Myristoleic acid C14:1 | 0.04 ± 0.01$^{a}$ | 0.12 ± 0.03$^{d}$ | 0.04 ± 0.01$^{a}$ | 0.05 ± 0.01$^{ab}$ | 0.03 ± 0.01$^{a}$ | 0.08 ± 0.02$^{c}$ | 0.13 ± 0.04$^{d}$ |
| Pentadecylic acid C15:0 | 0.35 ± 0.04$^{bc}$ | 0.37 ± 0.08$^{bc}$ | 0.48 ± 0.12 $^{cd}$ | 0.59 ± 0.08$^{d}$ | 0.33 ± 0.08$^{a}$ | 0.38 ± 0.05$^{bc}$ | 0.42 ± 0.06$^{bc}$ |
| Ginkgolic acid C15:1 | 0.06 ± 0.01$^{a}$ | 0.08 ± 0.02$^{a}$ | 0.10 ± 0.02$^{ab}$ | 0.13 ± 0.04$^{b}$ | 0.07 ± 0.02$^{a}$ | 0.06 ± 0.02$^{a}$ | 0.09 ± 0.02$^{ab}$ |
| Palmitic acid C16:0 | 16.06 ± 0.14$^{e}$ | 15.96 ± 0.21$^{de}$ | 14.43 ± 0.21$^{b}$ | 12.97 ± 0.12$^{a}$ | 15.45 ± 0.16$^{c}$ | 15.74 ± 0.12$^{d}$ | 13.23 ± 0.14$^{a}$ |
| Palmitoleic acid C16:1 | 0.13 ± 0.02$^{abc}$ | 0.13 ± 0.02$^{abc}$ | 0.17 ± 0.04 $^{cd}$ | 0.20 ± 0.02$^{d}$ | 0.12 ± 0.02$^{ab}$ | 0.10 ± 0.02$^{a}$ | 0.15 ± 0.04$^{bc}$ |
| Heptadecylic acid C17:0 | 1.83 ± 0.05$^{b}$ | 2.18 ± 0.07 $^{cd}$ | 1.65 ± 0.04$^{a}$ | 1.83 ± 0.07$^{b}$ | 2.21 ± 0.07$^{d}$ | 2.19 ± 0.07 $^{cd}$ | 2.09 ± 0.06$^{c}$ |
| Heptadecenoic acid C17:1 | 1.42 ± 0.03$^{d}$ | 0.18 ± 0.02$^{a}$ | 0.18 ± 0.03$^{a}$ | 0.18 ± 0.02$^{a}$ | 0.38 ± 0.03$^{b}$ | 2.17 ± 0.04$^{e}$ | 1.19 ± 0.03$^{c}$ |
| Stearic acid C18:0 | 18.68 ± 0.23$^{d}$ | 18.09 ± 0.11$^{c}$ | 17.73 ± 0.15$^{b}$ | 17.23 ± 0.12$^{a}$ | 18.08 ± 0.15$^{c}$ | 17.38 ± 0.18$^{a}$ | 17.28 ± 0.14$^{a}$ |
| Oleic acid C18:1 | 18.93 ± 0.21$^{a}$ | 19.62 ± 0.17$^{b}$ | 19.64 ± 0.18$^{b}$ | 20.13 ± 0.16$^{c}$ | 21.13 ± 0.18$^{d}$ | 21.92 ± 0.19$^{e}$ | 22.34 ± 0.17$^{f}$ |
| Linoleic acid C18:2 | 3.73 ± 0.08$^{a}$ | 4.83 ± 0.09$^{b}$ | 5.79 ± 0.11$^{d}$ | 5.83 ± 0.09$^{d}$ | 5.31 ± 0.11$^{c}$ | 5.34 ± 0.09$^{c}$ | 5.33 ± 0.10$^{c}$ |
| Gamma-linolenic acid C18:3(n-6) | 0.18 ± 0.01$^{a}$ | 0.22 ± 0.01$^{b}$ | 0.32 ± 0.02$^{d}$ | 0.29 ± 0.01$^{c}$ | 0.17 ± 0.02$^{a}$ | 0.18 ± 0.01$^{a}$ | 0.21 ± 0.01$^{b}$ |
| alpha-linolenic acidC18:3 (n-3) | 0.68 ± 0.03$^{ab}$ | 0.70 ± 0.04$^{abc}$ | 0.91 ± 0.05$^{e}$ | 0.75 ± 0.03 $^{cd}$ | 0.66 ± 0.03$^{a}$ | 0.73 ± 0.04$^{bc}$ | 0.81 ± 0.04$^{d}$ |
| Arachidic acid C20:0 | 2.06 ± 0.08$^{f}$ | 1.98 ± 0.06$^{f}$ | 0.41 ± 0.01$^{c}$ | 0.32 ± 0.01$^{b}$ | 1.82 ± 0.03$^{e}$ | 1.08 ± 0.02$^{d}$ | 0.08 ± 0.01$^{a}$ |
| Eicosenoic acid C20:1n9 | 0.42 ± 0.01$^{ab}$ | 0.48 ± 0.02 $^{cd}$ | 0.57 ± 0.02$^{e}$ | 0.51 ± 0.04$^{d}$ | 0.41 ± 0.02$^{a}$ | 0.46 ± 0.03$^{bc}$ | 0.60 ± 0.02e |
| Eicosadienoic acid C20:2 | 9.84 ± 0.06$^{a}$ | 11.07 ± 0.11$^{b}$ | 11.34 ± 0.13$^{c}$ | 12.86 ± 0.12$^{d}$ | 12.87 ± 0.12$^{d}$ | 14.37 ± 0.14$^{e}$ | 14.95 ± 0.16$^{f}$ |
| Arachidonic acidC20:4 (n-6) | 0.88 ± 0.02$^{c}$ | 0.74 ± 0.02$^{b}$ | 1.02 ± 0.03$^{d}$ | 1.04 ± 0.03$^{d}$ | 0.37 ± 0.01$^{a}$ | 0.34 ± 0.01$^{a}$ | 1.16 ± 0.02$^{e}$ |
| Eicosapentaenoic acidC20:5 (n-3) | 1.34 ± 0.03$^{b}$ | 1.11 ± 0.02$^{a}$ | 1.66 ± 0.04$^{e}$ | 1.56 ± 0.03$^{d}$ | 1.50 ± 0.04$^{d}$ | 1.42 ± 0.03$^{c}$ | 1.88 ± 0.03$^{f}$ |

* Values are given as mean ± SD (standard deviation).
** Different letters in the same row indicate statistically significant differences at P = 95 % confidence level according to Fisher's least significant difference (LSD) procedure.

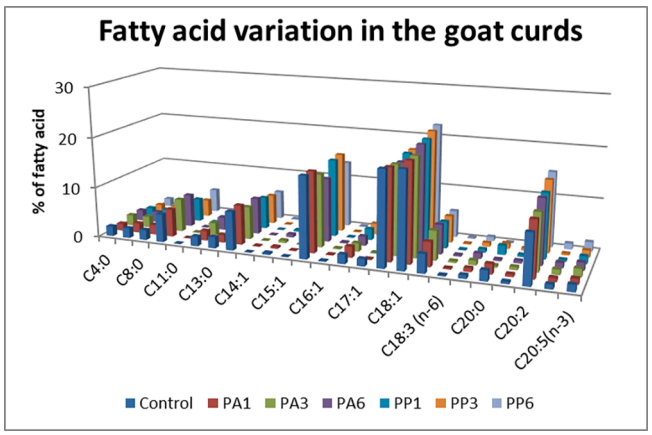


**Fig. 8.** Fatty acids variation in the goat curds.

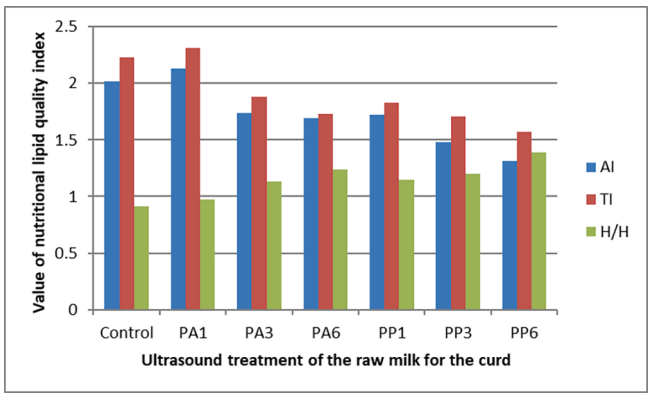


**Fig. 9.** Nutritional lipid quality indices of curds prepared from the ultrasound-treated goat milk.

from the milk treated for shorter periods of time registered higher acidity values because they were characterized by a higher number of microorganisms that could generate more acidity.

### 3.5. Fatty acids profile

Lipids fatty acids composition of the curds obtained from the goat milk subjected to different power-time ultrasound regimes of pasteurization compared with the curd obtained from the control goat milk (untreated) are shown in Table 4.

Twenty-five fatty acids were identified in the curd samples with C4 to C20 carbon. The fatty acids were saturated fatty acids (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA). Myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0) were found to be the major SFA in all goat curds while between monounsaturated fatty acid, oleic acid (C18:1) was found at the highest levels (Fig. 8).

An increase in the unsaturated fatty acids for the curds obtained from the milk ultrasound treated was observed. Van Hekken et al. [49] reported a slight increase of conjugated linoleic acid C18:2 trans, trans following 14 to 18 min of ultrasound treatment of cow milk. Sergeev et al. [50] reported that the fatty acids profile of goat milk from the butter obtained by ultrasound-treated milk and the untreated milk is unchanged. The goat milk was subjected to a frequency of 45 kHz with an energy exposure of only ~6 W/L. In the present study, the frequency of ultrasounds was 40 kHz, but the power was higher: 320 W and 881 W when ultrasound treatment favors lipolysis and results in enhanced heat and mass transfer [13]. Unsaturated fatty acids were probably formed due to the ultrasound treatment that promotes the destruction of the lipoprotein membrane of the fat globules with the release of lipids which are then broken down by lipolysis into triglycerides and further to fatty acids [51].

### 3.6. Lipid quality indices in curds

The values of AI ranged from 1.29 to 2.09 and showed a decreasing trend with the increase of the power and time of the ultrasound treatment of goat milk. Lower values of the AI index are desirable. Paszczyk and Luczynska [28] reported AI values for goat cheese in the range of 1.12–2.14. Dauber et al. [52] reported an AI value of 3.08 for the lipids in goat cheese. The goats were fed with concentrates. The lipid profile of milk was modified, and the AI value of goat cheese decreased to 2.59 when the goats' diet was supplemented with sunflower.

TI values of the lipids extracted from the curds varied between 1.57 and 2.31(Table 5). TI value shows the tendency for blood clot formation in the blood vessels as it shows the relationship between the prothrombogenic (SFA) and the anti-thrombogenic fatty acids (MUFA n-3 and n-6 PUFA) [28]. Low TI values are related to a lower risk of blood vessel affections. Paszczyk and Luczynska [28] reported an average TI value for goat cheese of about 2.67, lower than the TI of lipids extracted from cow and sheep cheeses.

The H/H ratio quantifies the functional activity of fatty acids in the metabolism of lipoproteins for plasma cholesterol transport [28] being desirable for a higher value. The curds showed H/H values that varied in the range of 0.86 and 1.17 with the highest value for the curd prepared from milk treated for 3 min at an ultrasound power of 881 W.

The different power-time ultrasound regimes of the milk pasteurization applied for the curds production seem to improve the values of the lipid quality indices (Fig. 9).

## 4. Conclusions

In this study the raw goat milk was treated with beta-cyclodextrin in the proportion of 0.6 %, leading to a cholesterol reduction of 92.1 %, and then subjected to different power-time ultrasound regimes of pasteurization. The curds produced by the US-treated milk processing were more appreciated from the organoleptic point of view compared to the control curd. Also, the PA6 and PP6 curds were characterized by the highest dry matter values due to an accentuated syneresis process. The acidity values for the PA1, PA3, and PA6 curds were higher because of more pronounced lactose hydrolysis. Twenty-five fatty acids were identified in the curds obtained by different power-time ultrasound regimes of pasteurization with C4 to C20 carbon and the unsaturated fatty acids proportion increased as the US regimes intensified. Ultrasound treatment could be a valid method to improve the microbiological quality of

**Table 5**
Values of the nutritional lipid quality indices of the curds obtained from goat milk treated to various power-time ultrasound regimes.

| Lipid quality indices * | Control | PA1 | PA3 | PA6 | PP1 | PP3 | PP6 |
|---|---|---|---|---|---|---|---|
| AI | $1.99 \pm 0.05^{d}$ | $2.09 \pm 0.07^{e}$ | $1.71 \pm 0.06^{c}$ | $1.67 \pm 0.05^{c}$ | $1.68 \pm 0.06^{c}$ | $1.46 \pm 0.05^{b}$ | $1.29 \pm 0.03^{a}$ |
| TI | $2.23 \pm 0.03^{e}$ | $2.31 \pm 0.01^{f}$ | $1.87 \pm 0.02^{d}$ | $1.85 \pm 0.01^{cd}$ | $1.83 \pm 0.03^{c}$ | $1.68 \pm 0.03^{b}$ | $1.57 \pm 0.02^{a}$ |
| H/H | $0.89 \pm 0.01^{a}$ | $0.97 \pm 0.02^{b}$ | $1.10 \pm 0.03^{c}$ | $1.18 \pm 0.03^{d}$ | $1.13 \pm 0.01^{c}$ | $1.18 \pm 0.01^{d}$ | $1.36 \pm 0.01$e |

**Different letters (a-e) in the same row indicate statistically significant differences at a P = 95 % confidence level according to Fisher's least significant difference (LSD) procedure.

* Values are given as mean ± SD (standard deviation).

goat cheese as the sonication significantly reduced the growth of mesophilic aerobic bacteria, E. coli positive for beta-glucuronidase, and coagulase-positive staphylococci (*S. aureus*) in the final products. The highest inactivation rate was observed in the samples treated for 6 min at 881 W. Also, ultrasound pasteurization of milk during curds production improved the nutritional indices of the lipids such as the index of atherogenicity, index of thrombogenicity, and the ratio of hypocholesterolemic and hypercholesterolemic fatty acids.

Regarding the widening of this field of research, it is necessary to deepen the aspects through which different conditions of ultrasound treatment affect the microstructure of a wide range of dairy products. Also, deeper knowledge is needed concerning the ultrasound influence on the food sensory characteristics, especially studies focused on proteolysis, lipolysis, off-flavors, and of course the degree of likeness with the greatest impact on food quality. Future studies can rely upon the use of ultrasounds in functional dairy products production, especially for bioactive components generation and enzymatic hydrolysis.

Future limits might be represented by the scale-up of this green ultrasound technology from the laboratory to industry respecting all the quality aspects because the amount of the raw material is very high. In this regard, the solution can be represented by continuous systems with the ultrasonic probe, ultrasonic baths with a larger radiating surface, or more ultrasonic transducers with an agitation system.

## 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.

## Data availability

Data will be made available on request.

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## References

[1] F. Yangilar, As a potentially functional food: Goats' milk and products, Journal of Food and Nutrition Research 1 (2013) 68–81, https://doi.org/10.12691/JFNR-1-4-6.
[2] P.C. Pereira, Milk nutritional composition and its role in human health, Nutrition 30 (6) (2014) 619–627, https://doi.org/10.1016/j.nut.2013.10.011.
[3] Y.W. Park, M. Juárez, M. Ramos, G.F.W. Haenlein, Physico-chemical characteristics of goat and sheep milk, Small Ruminant Research 68 (1-2) (2007) 88–113.
[4] L. Alonso, P. Fox, M. Calvo, J. Fontecha, Effect of beta cyclodextrin on the reduction of cholesterol in ewe's milk Manchego cheese, Molecules 23 (7) (2018) 1789, https://doi.org/10.3390/molecules23071789.
[5] A.M. Maskooki, S.H.R. Beheshti, S. Valibeigi, J. Feizi, Effect of cholesterol removal processing using β-cyclodextrin on main components of milk, International Journal of Food Science 5 (2013) 1–6, https://doi.org/10.1155/2013/215305.
[6] L. Alonso, J. Fontecha, P. Cuesta, Effect of β-Cyclodextrin on Phospholipids and Cholesterol of the Milk Fat Globule Membrane, J Adv Dairy Res, 3 (3) (2015), pp. 140. https://doi.org/10.4172/2329-888X.1000140.
[7] https://foodcrafters.org/products/foodcrafting-in-romania-milk/ (accessed 13 August 2013).
[8] L. Zhao, S. Zhang, H. Uluko, L. Liu, J. Lu, H. Xue, F. Kong, J. Lv, Effect of ultrasound pretreatment on rennet-induced coagulation properties of goat's milk, Food Chemistry 165 (2014) 167–174, https://doi.org/10.1016/j.foodchem.2014.05.081.
[9] F. Remeuf, Physico-chemical properties of goat milk in relation to processing characteristics. In V International conference on goats: March 1992 preconference proceedings: Invited papers. Part 2, 2 (1992), pp. 374–381.
[10] F. Remeuf, J. Lenoir, Relationship between the physico-chemical characteristics of goat's milk and its rennetability, International Dairy Bull 202 (1986) 68–72.
[11] G.E. Vegarud, T. Langsrud, M.J. Brovold, T.G. Devold, B.O. Henriksen, J. Oyaas, P. Alestrom, S. Lien, T. Steine, S. Rogne, Cow's milk protein genotypes: quality and stability of raw milk, pasteurized milk and fermented milk, International Dairy Journal 9 (1999) 399–400, https://doi.org/10.1016/S0958-6946(99)00105-3.
[12] Z. Liu, P. Juliano, R.P.W. Williams, J. Niere, M.A. Augustin, Ultrasound improves the renneting properties of milk, Ultrasonics Sonochemistry 21 (2014) 2131–2137, https://doi.org/10.1016/j.ultsonch.2014.03.034.
[13] L.M. Carrillo-Lopez, I.A. Garcia-Galicia, J.M. Tirado-Gallegos, R. Sanchez-Vega, M. Huerta-Jimenez, M. Ashokkumar, A.D. Alarcon-Rojo, Recent advances in the application of ultrasound in dairy products: Effect on functional, physical, chemical, microbiological and sensory properties, Ultrasonics Sonochemistry 73 (2021) 1–35, https://doi.org/10.1016/j.ultsonch.2021.105467.
[14] H. Scudino, E. K. Silva, A. Gomes, J. T. Guimarães, R. L. Cunha, A. S. Sant'Ana, M. Angela, A. Meireles, A, G. Cruz, Ultrasound stabilization of raw milk: Microbial and enzymatic inactivation, physicochemical properties and kinetic stability, Ultrasonics Sonochemistry, 67 (2020), pp. 1-11. doi: 10.1016/j.ultsonch.2020.105185.
[15] A. Patist, D. Bates, Ultrasonic innovations in the food industry: From the laboratory to commercial production, Innovative Food Science & Emerging Technologies 9 (2) (2008) 147–154, https://doi.org/10.1016/j.ifset.2007.07.004.
[16] M. Ashokkumar, R. Bhaskaracharya, S. Kentish, J. Lee, M. Palmer, B. Zisu, The ultrasonic processing of dairy products – an overview, Dairy Sci. Technol. 90 (2) (2009) 147–168, https://doi.org/10.1051/dst/2009044.
[17] J. Chandrapala, T. Leong, Ultrasonic processing for dairy applications: recent advances, Food Eng. Rev. 7 (2) (2015) 143–158, https://doi.org/10.1007/s12393-014-9105-8.
[18] J.T. Guimarães, E.K. Silva, M.Q. de Freitas, M.A.A. Meireles, A.G. da Cruz, Nonthermal emerging technologies and their effects on the functional properties of dairy products, Curr. Opin. Food Sci. 22 (2018) 62–66, https://doi.org/10.1016/j.cofs.2018.01.015.
[19] J.T. Guimarães, E.K. Silva, C.S. Ranadheera, J. Moraes, R.S.L. Raices, M.C. Silva, M. S. Ferreira, M.Q. Freitas, M.A.A. Meireles, A.G. Cruz, Effect of high-intensity ultrasound on the nutritional profile and volatile compounds of a prebiotic soursop whey beverage, Ultrasonics Sonochemistry 55 (2019) 157–164, https://doi.org/10.1016/j.ultsonch.2019.02.025.
[20] S.H.M.C. Monteiro, E.K. Silva, V. O. Alvarenga, J. Moraes, J., M. Q. Freitas, M. C, Silva, R. S. L. Raices, A. S. Sant'Ana, M. Meireles, A. G. Cruz, Effects of ultrasound energy density on the non-thermal pasteurization of chocolate milk beverage, Ultrasonics Sonochemistry, 42 (2020), 1-10, DOI: 10.1016/j.ultsonch.2017.11.015.
[21] F. Chemat, M.K. Zill-E-Huma, M.K. Khan, Applications of ultrasound in food technology: processing, preservation and extraction, Ultrasonics Sonochemistry 18 (4) (2011) 813–835, https://doi.org/10.1016/j.ultsonch.2010.11.023.
[22] A. Dumuta, C. Mihali, Z. Berinde, Z. Vosgan, L. Mihalescu, A. Noghi, Milk cholesterol reduction at pilot station level using beta-cyclodextrin, Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Food Science and Technology 77 (2) (2020) 82–87, https://doi.org/10.15835/buasvmcn-fst: 2020.0005.
[23] M. Stanciu, C. Sand, G. Săvoiu, I. Vlad, M. Antofie, I. Antonie, G. Bahaciu, V. Băcilă, M. Bănuță, R. Blaj, N. Caragea, G. Ciortea, V. Crăciuneanu, V. Deleanu, G. Diniță, V. Firescu, P. Iagăru, A. Pavelescu, M. Pomohaci, G. Purima, L. Vidu, M. Ureche, Studiu privind stâna, produsele şi subprodusele tradiționale de stână din arealul rural montan , 2010. Editura Universității" Lucian Blaga" din Sibiu.
[24] ISO 4833:2003 – Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 degrees C.
[25] ISO 16649-2:2001 – Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of β-glucuronidase-positive Escherichia coli Colony-count technique at 44°C.
[26] ISO 6888-1:2021 – Microbiology of the food chain – Horizontal method for the enumeration of coagulasepositive staphylococci (Staphylococcus aureus and other species) – Part 1: Method using Baird-Parker agar medium.
[27] A. Dumuta, Z. Vosgan, Analiza (senzoriala, fizico-chimica, microbiologică) şi controlul calității în industria laptelui şi produselor lactate. Ghid practic, 2015. Ed. U.T. Press, Cluj Napoca.
[28] B. Paszczyk, J. Łuczynska, The Comparison of Fatty Acid Composition and Lipid Quality Indices in Hard Cow, Sheep and Goat Cheeses, Foods 9 (11) (2020) 1667, https://doi.org/10.3390/foods9111667.
[29] J. Chen, H. Liu, Review Nutritional Indices for Assessing Fatty Acids: A Mini-Review, International Journal of Molecular Sciences 21 (2020) 5695, https://doi.org/10.3390/ijms21165695.
[30] S. Ivanova, Lj. Angelov, Assessment of the Content of Dietary Trans Fatty Acids and Biologically Active Substances in Cow's Milk and Curd, Modern Chemistry 2017 5 (6) (2017), pp. 86-92, DOI: 10.11648/j.mc.20170506.11.
[31] T. Ulbricht, D. Southgate, Coronary heart disease: Seven dietary factors, Lancet 338 (1991) 985–992, https://doi.org/10.1016/0140-6736(91)91846-m.
[32] T.A. Hernández-Falcón, A. Araceli Monter-Arciniega, N. del S. Cruz-Cansino, E. Alanís-García, G.M. Rodríguez-Serrano, A. Castañeda-Ovando, M. García-Garibay, E. Ramírez-Moreno, J. Jaimez-Ordaz, Effect of thermoultrasound on aflatoxin M1 levels, physicochemical and microbiological properties of milk during storage, Ultrasonics Sonochemistry, 48 (2018), pp. 396-403. doi: 10.1016/j.ultsonch.2018.06.018.
[33] D. Bermúdez-Aguirre, R. Mawson, G.V. Barbosa-Canovas, Microstructure of fat globules in whole milk after thermosonication treatment, J. Food Sci. 73 (2008) 325–332, https://doi.org/10.1111/j.1750-3841.2008.00875. x.
[34] S. Karlovic, T. Bosiljkov, M. Brnčic, D. Semenski, F. Dujmic, B. Tripalo, D. Ježek, Reducing fat globules particle-size in goat milk: ultrasound and high hydrostatic

pressures approach, Chem. Biochem. Eng. Q. J., 28 (2015), pp. 499–507. https://doi. org/10.15255/cabeq.2014.19400.
[35] A. Shanmugam, J. Chandrapala, M. Ashokkumar, The effect of ultrasound on the physical and functional properties of skim milk, Innovative Food Science & Emerging Technologies 16 (2012) 251–258, https://doi.org/10.1016/j.ifset.2012.06.005.
[36] Q. Wu, X. Zhang, J. Jia, C. Kuang, H. Yang, Effect of ultrasonic pretreatment on whey protein hydrolysis by alcalase: thermodynamic parameters, physicochemical properties and bioactivities, Process Biochem. 67 (2018) 46–54, https://doi.org/10.1016/j.procbio.2018.02.007.
[37] V. Akdeniz, A.S. Akalin, New approach for yoghurt and ice cream production: high-intensity ultrasound, Trends Food Sci. Technol. 86 (2019) 392–398, https://doi.org/10.1016/j.tifs.2019.02.046.
[38] D.K. Lee, J. Ahn, H.S. Kwak, Cholesterol removal from homogenized milk with β-cyclodextrin, Journal of Dairy Science 82 (11) (1999) 2327–2330.
[39] L. Alonso, P. Cuesta, J. Fontecha, M. Juarez, S.E. Gilliland, Use of β-cyclodextrin to decrease the level of cholesterol in milk fat, J. Dairy Sci. 92 (3) (2009) 863–869, https://doi.org/10.3168/jds.2008-1452.
[40] E. Christoforides, A. Papaioannou, K. Bethanis, Crystal structure of the inclusion complex of cholesterol in β-cyclodextrin and molecular dynamics studies, Beilstein Journal of Organic Chemistry 14 (2018) 838–848, https://doi.org/10.3762/bjoc.14.69.
[41] A. Jalilzadeh, J. Hesari, S.H. Peighambardoust, I. Javidipour, The effect of ultrasound treatment on microbial and physicochemical properties of Iranian ultrafiltered feta-type cheese, J. Dairy Sci. 101 (2018) 5809–5820, https://doi.org/10.3168/jds.2017-14352.
[42] S. Gao, G.D. Lewis, M. Ashokkumar, Y. Hemar, Inactivation of microorganisms by low-frequency high-power ultrasound: 2, A simple model for the inactivation mechanism, Ultrasonics Sonochemistry 21 (1) (2013) 454–460, https://doi.org/10.1016/j.ultsonch.2013.06.007.
[43] Z. Herceg, E. Juraga, B. Sobota-Šalamon, A. Rezek-Jambrak, Inactivation of mesophilic bacteria in milk by means of high intensity ultrasound using response surface methodology, Czech, J. Food Sci. 30 (2012) 108–117, https://doi.org/10.17221/93/2011-CJFS.
[44] J. Chandrapala, C. Oliver, S. Kentish, M. Ashokkumar, Ultrasonics in food processing—Food quality assurance and food safety, Trends Food Sci. Technol. 26 (2012) 88–98, https://doi.org/10.1016/j.tifs.2012.01.010.
[45] L.M. Carrillo-Lopez, M.G.M.G. Juarez-Morales, I.A. Garcia-Galicia, A.D.A. D. Alarcon-Rojo, M. Huerta-Jimenez, The effect of high-intensity ultrasound on the physicochemical and microbiological properties of Mexican panela cheese, Foods. 9 (2020) 1–14, https://doi.org/10.3390/foods9030313.
[46] M. Cameron, L.D. Mcmaster, T.J., Britz, Impact of ultrasound on dairy spoilage microbes and milk components, Dairy Sci. Technol., 89 (2009), pp. 83–98. DOI: 10.1051/dst/2008037.
[47] N.H.A. Nguyen, S.G. Anema, Ultrasonication of reconstituted whole milk and its effect on acid gelation, Food Chem. 217 (2017) 593–601, https://doi.org/10.1016/j.foodchem.2016.08.117.
[48] A.K. Niamah, Ultrasound treatment (low frequency) effects on probiotic bacteria growth in fermented milk, Futur. Food J. Food, Agric. Soc., 7 (2019), pp. 1–8. DOI: 10.17170/kobra-20190709592.
[49] D.L. Van Hekken, J. Renye Jr., A.J. Bucci, P.M. Tomasula, Characterization of the physical, microbiological, and chemical properties of sonicated raw bovine milk, Journal of Dairy Sciences 102 (2019) 6928–6942, https://doi.org/10.3168/jds.2018-15775.
[50] A. Sergeev, N. Shilkina, M. Motyakin, I. Barashkova, V. Zaborova, K. Kanina, N. Dunchenko, O. Krasulya, N.S.M. Yusof, Anhydrous fat crystallization of ultrasonic treated goat milk: DSC and NMR relaxation studies, Ultrasonics Sonochemistry 78 (2021) 1–7, https://doi.org/10.1016/j.ultsonch.2021.105751.
[51] H. Feng, G.V. Barbosa-Cánovas, J. Weiss (eds.), Ultrasound Technologies for Food and Bioprocessing, Food Engineering Series, 445 (2011), Springer Science + Business Media, pp. 456-462. https://doi.org/10.1007/978-1-4419-7472-3_16.
[52] C. Dauber, T. Carreras, A. Britos, S. Carro, C. Cajarville, A. Gambaro, S. Jorcin, T. Lopez, I. Vieitez, Elaboration of goat cheese with increased content of conjugated linoleic acid and transvaccenic acid: Fat, sensory and textural profile, Small Ruminant Research., 199 (2021), pp. 1-11. 106379, https://doi.org/10.1016/j.smallrumres.2021.106379.