Reducing Fat Globules Particle-Size in Goat Milk: Ultrasound and High Hydrostatic Pressures Approach

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doi: 10.15255/CABEQ.2014.19400

Original scientific paper
Received: September 9, 2014
Accepted: December 8, 2014

Innovative and eco-friendly food technologies in practical usage today like Ultrasound (US) and High Hydrostatic Pressures (HHP) are feasible to adequately maintain various food properties while processed, such as texture, sensorial and organoleptic characteristics, and microbiological issues as well. Benchmarked attribute of the mentioned approach lies in the ability of US and HHP to control and withhold both temperature and treatment duration. While temperature could be controlled within room ambient, treatment time is mostly below 30 minutes. US and HHP treatment were performed as separate treatments in order to obtain better homogenization. Goat milk was exposed to ultrasonic propagation up to 100 W of nominal power and high pressures up to 600 MPa. Maximum treatment time was 9 minutes. Ultrasonic homogenization indicates enhanced homogeneity of fat globules while high pressure process parameters have a significant influence on the observed mean particle diameter (fat globules). Improved stability and quality of emulsions (goat milk) was obtained by both applied processes. Statistical analysis indicated the influence of process parameters on fat globule size distribution between 0.3 – 4 μm and variance lower than 0.6.

Key words:

goat milk, ultrasound, high hydrostatic pressure, homogenization, fat globules

Introduction

Advantages of goat milk over cow milk

In milk processing, special emphasis is directed to fostering the taste and healthy values of goat milk. Therapeutic properties of goat milk are very well known. Comparison of cow and goat milk at the level of chemical composition suggests significant differences in their physical and chemical characteristics. Smaller diameter of fat globules (natural homogeneity), high portion of essential amino acids and soluble minerals lead to higher digestibility of goat milk in comparison with cow milk. Goat milk has a large total area of fat globules compared with cow milk. Due to lower diameter of fat globules, the velocity of fat skimming is faster, indicating less energy consumption during processing.[1,2]

Ultrasound and high hydrostatic pressure processing

High intensity ultrasound of intensities above 1 W cm^{-2} can cause physical and chemical changes in inhomogeneous goat milk. The main goal of ultrasonic homogenization is the reduction of fat globule size.[3,4] The most significant effect of ultrasonic treatment is increasing of stability and consistency of obtained emulsion. Separation of fat and water phases is not possible during consummation and storage over a period of time. The term "homogenization" represents the processes that include the heterogeneous dispersed system. Finally, achieved is a higher dispersion in the system of different relative frequency of milk fat globule distribution. Efficiency of ultrasound is based on fat globule disruption in an oil-in-water system as a direct consequence of cavitation effect. Implementation of higher amplitude and longer treatment time lead to formation of a large number of smaller fat globules.[5] Observation and analysis of samples treated in such way could be very difficult, especially in the scope of defining the interval of fat globules. Emulsions are thermodynamically unstable and their stability may be improved by adding surface-active substances and emulsifiers. Dispersion and absorption of the ultrasonic wave into liquid media transforms the mechanical into heat energy, thus achieving an increase in the sample's temperature. Different mechanisms have a significant influence on absorption of ultrasonic wave.

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The most efficient are variation in viscosity and thermal conductivity, as a consequence of internal friction among molecules.[6,7]

The initial power burst of ultrasonic wave, starting amplitude, as well as the ultrasonic wave propagating through the liquid medium, can be absorbed and dissipated. This kind of appearance can be represented by the attenuation coefficient (α) shown in eq. (1):[8–11]

$$\alpha = \frac{\mu \cdot \omega^2}{2 \cdot c_v^3 \cdot \rho} \quad (1)$$

where: α – attenuation coefficient [Neper m^{-1}] μ – dynamic viscosity [Pa s]; ω – nominal frequency of selected probe [Hz]; c_v – propagation velocity of ultrasonic wave [m s^{-1}]; ρ – density [kg m^{-3}].

Homogenization under high pressure and ultrasound can influence the desirable physical and chemical properties of food products. The most relevant are changes in density, viscosity, pH, emulsion stability index, absorbance and temperature as a consequence of propagation of ultrasonic wave or high pressures applied on liquid medium during processing.[12–15] The mechanism of transient cavitation can produce shock waves that increase the temperature in the surrounding media, in fact, the mechanism is based on the implosion of formed cavitation bubbles. They exist for a very short time, less than one cycle, and collapse rapidly and violently, which immediately increases the temperature in close proximity to approximately 5000 K, while pressure exceeds 100 MPa.[16–20] Analyses of covariance give us the result of produced effects of temperature on changes of all observed physical properties.[8] After the ultrasonic homogenization at maximum value of process parameters, the temperature of the goat milk reached 50 °C.[21] This temperature value has no influence on the variation of protein conformation (denaturation) or the chemical composition of milk.[22–24] By controlling the temperature and reducing the homogenization duration, it is possible to avoid negative effects of sonication, such as lipid oxidation.[25,26] Today, high pressure processing is used for the treatment of meat products (inactivation of microorganisms), pasteurization of juice and tomato juices, freezing and defrosting of various food materials, homogenization and modification of physical characteristics of dairy products (e.g. foaming). The main purpose of high pressure treatment is preservation of nutritive and textural properties.[27] The mechanism of the direct method of high pressure processing is based on transportation of high pressure oil over the surface of a piston. The piston exerts pressures of approximately 20 MPa on the – pressure-transmitting liquid (water, propylene – glycol) which is introduced to compress the food material. The indirect method is based on pumping up the – pressure-transmitting fluid into a high pressure vessel. The driving pressure is distributed uniformly throughout the surface of the product independently of its quality and shape. Thermal characteristics of the composition of pressure-transmitting fluids to food material play a very important role in controlling the thermal behavior of food materials under high pressure. For liquids (milk, juices), at every 100 MPa the temperature increases by 2.6 – 3.0 °C during pressurizing in a vessel.[28] Maximum working pressure is inversely proportional to the volume of the working cylinder in all industrial and laboratory high – pressure equipment. Maximum pressures applied in the food processing industry range from 200 – 1000 MPa. Holding time depends on the physical and chemical properties of the processed material and it is usually adjusted up to 30 minutes.[29,30] Compared to classic milk homogenizers which work at 18 MPa, an increase in pressure of to up to 200 MPa produces a larger number of smaller fat globules.[31] Milk treated with pressures from 400 MPa, for a long time, such as 30 minutes, undergo psychical changes such as protein hydrophobicity, changes in pH, decrease in average diameter of particles and altered rennet coagulation as an important functional change.[32] Improved physical properties of high hydrostatic pressure homogenized milk, retained color and decrease in microbial count could enhance the quality of dairy products such as cheeses and yogurts.[33] Smaller pressures (up to 150 MPa) and shorter processing times have no such influence on the psychical characteristics, but high hydrostatic pressure processing which starts from 200 MPa still leads to decrease in particle size diameter of casein micelles.[34]

Materials and methods

Sample

Inhomogeneous goat milk (marked as 0) with following chemical composition is described with related Mean particle diameters [μm]:

Protein content (N×6.25): 2.7 %; Total sugars: 4.1 %; Fat content: 3.7 %; Minerals: Ca – 960 mg kg^{-1} P – 680 mg kg^{-1}.

D (0,1) = 0.158; D (0,5) = 2.562; D (0,9) = 5.384; D (3,2) = 0.516; D (4,3) = 2.603

Initial sample temperature was 20 °C.

Ultrasonic homogenization

Ultrasonic processor (*UP 100 H; Hielscher, Teltow, Germany*) with maximum nominal power of 100 W was used for ultrasonic homogenization of obtained samples. Volume of the treated sample was 250 mL. Constant ultrasonic frequency was 30 kHz, amplitude 60 and 100 % with full duty cycle over the entire treatment period which was 3, 6 and 9 minutes. Diameter of the probes was 7 and 10 mm. The energy density was calculated using eq. 2:

$$\text{Energy density}\left[\text{J mL}^{-1}\right] = \\ = \frac{\text{Power drawn}\left[\text{W}\right] \cdot \text{Time}\left[\text{s}\right]}{\text{Volume}\left[\text{mL}\right]} \quad (2)$$

High hydrostatic pressure homogenization

High hydrostatic pressure processing (*Stansted Fluid Power, Great Britain*) was performed with pressures of 200, 400 and 600 MPa, holding time (time of treatment) was 3, 6 and 9 minutes. Volume of the treated sample was 250 mL. Sample was poured in plastic bottle and placed in the working vessel with maximum capacity of 2000 mL.

Measuring of absorbance and calculation of ESI (Emulsion Stability Index)

ESI was calculated over measured absorbance with spectrophotometer (*Conica – Minolta CM – 3500 d, Japan*) using eq. 3 and 4:

$$T = \frac{2.303 \cdot A_{500}}{d_c} \quad (3)$$

where: T – optical density; $A_{500\text{nm}}$ – absorbance; d_c – diameter of cuvette [m]

$$ESI = \frac{T \cdot \Delta t}{\Delta T} \quad (4)$$

where: *ESI* – Emulsion Stability Index [h]; Δt – time interval [24h]; ΔT – optical density variation over 24 hours.

Microscopy

High definition digital camera (*Olympus E – 520 KIT, Japan*) was attached on the C – mount of microscope (*Leica DM 1000 LED, Solms, Germany*). Pictures of fat globules were recorded with magnification of 1000× and analysed using "*Image – J" software.*

Fat globule size distribution

Fat globule distribution was measured by light scattering (0.02 – 140 °) through the working cell using external unit "*Hydro 2000 S*" (*Malvern Masterseizer 2000, Great Britain*). The volume of working cell was filled with 150 mL of distilled water, and 1 – 5 mL of sample was added to the volume, depending of the degree of obscuration. Refractive Index (n_r) of the sample was 1.338 and dispersant 1.333. Stirrer speed was 1850 rpm.

After measuring, the particle diameters were expressed over: D (3,2) – The area mean (Sauter mean diameter, according to eq. 5) – weighted average surface diameter, take in relation spherical particles of the same surface area as the measured particles – for interpretations of smaller particles (fat globules) in size of distribution. D (4,3) – The Volume moment mean (De Brouckere mean diameter, according to eq. 6) – weighted average volume diameter, take in relation spherical particles of the same volume as the measured particles – for interpretations of smaller particles (fat globules) in size of distribution They were represented with following expression where, (n) is the number of fat globules having a diameter [m] identical to (d_i).[35] Sphericity (Φ) is a term for unideal individual particles and can be defined by eq. 7.; where (x_p) is the equivalent diameter of one particle [m], (s_p) is the surface area of one particle [m^2], (V_p) is the volume of one particle [m^3].[36] D (0.1) – 10 % of the volume distribution is below observed diameter, consistently, D (0.9) – 90 % of the volume distribution is below observed diameter, D (0,5) – median diameter, 50 % of the volume distribution is above, and 50 % is below observed diameter. The *"span"* of fat globules can be calculated using eq. 8:

$$D[3,2] = \frac{\sum_i n_i \cdot d_i^3}{\sum_i n_i \cdot d_i^2} \quad (5)$$

$$D[4,3] = \frac{\sum_i n_i \cdot d_i^4}{\sum_i n_i \cdot d_i^3} \quad (6)$$

$$\Phi_S = \frac{6 \cdot V_p}{x_p \cdot s_p} \quad (7)$$

$$\text{Span} = \frac{D(0,9) - D(0,1)}{D(0,5)} \quad (8)$$

Statistical analysis

Statistical analysis was performed using "*Statistica 10" software*. Statistically significant influence of all process parameters of both non-thermal processes is expressed over p – values. *ANOVA (Analysis of Variance)* factors having $p < 0.05$, *(95 % – level of significance)* were taken into consideration. Positive or negative β *– coefficient* of applied process parameter indicates increasing or decreasing of observed mean particle diameter.

Results and discussion

Natural homogeneity as a special physical property of goat milk emphasizes a larger number of smaller fat globules present in inhomogeneous milk.

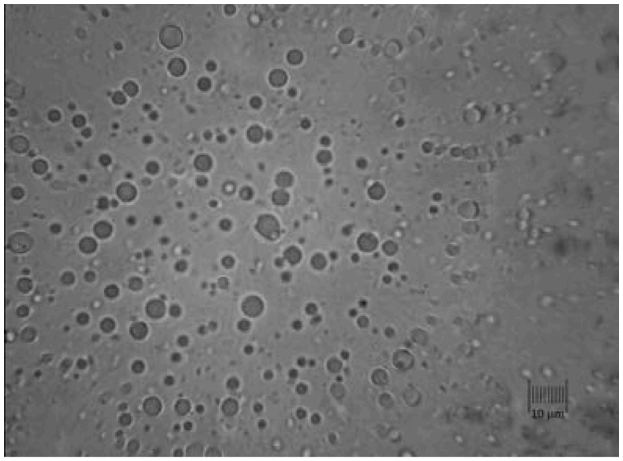
In order to adequately represent natural homogeneity of goat milk, transformation of frequency curve is necessary. The fat globule size distribution is normally presented with *Log – N distribution* (volume size distribution) (9):[37]

$$f(d) = \frac{1}{\sqrt{2 \cdot \pi} \cdot \ln \sigma} \exp\left(- \left[\frac{\ln d - \ln \overline{d}}{\sqrt{2} \cdot \ln \sigma} \right]^2 \right) \quad (9)$$

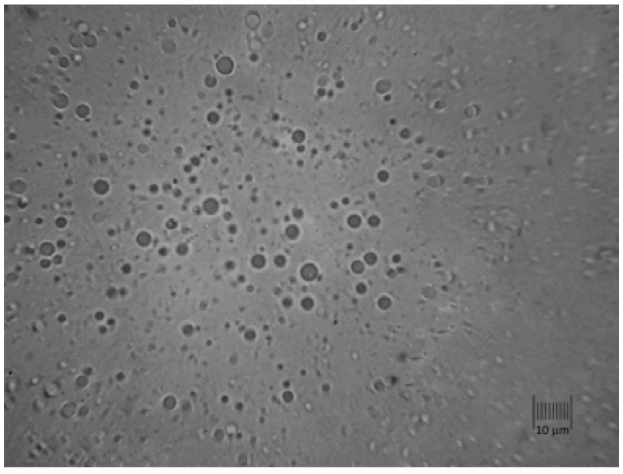
where: d – diameter of fat globules; $\overline{d}$ – mean value; σ – variance.

The primary results may be transformed into number weighted distribution. In such distribution, the number of smaller fat globules of goat milk is explicitly represented.

The microscopic image of inhomogeneous goat milk (Fig. 1 – a) shows that a large number of globules smaller than 1 μm are not clearly visible. Homogeneous goat milk after 9 minutes at 100 W is presented in Fig. 1 – b. The differences in the presentations of inhomogeneous goat milk and transformation of volume into number weighted distribution are shown in Figs. 2 and 3.



a



b

Fig. 1 – *Microscopic images (magnification 1000×) of inhomogeneous goat milk (a) and homogeneous (b) under 100 W and 9 minutes of sonication*

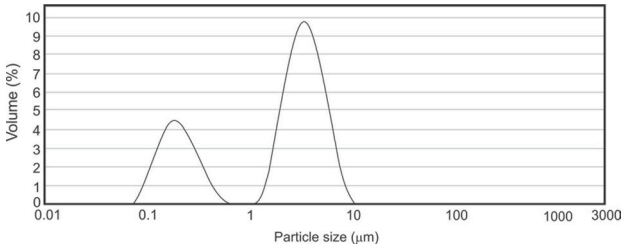


Fig. 2 – *Volume size distribution of inhomogeneous goat milk*

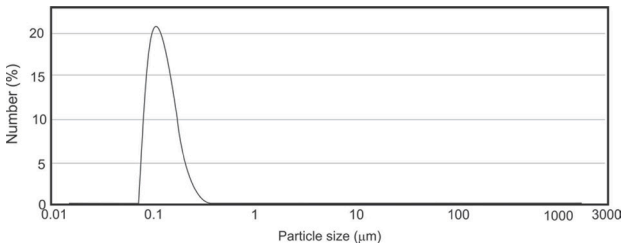


Fig. 3 – *Number size distribution of inhomogeneous goat milk*

As expected, high hydrostatic pressure and ultrasonic homogenization had a significant influence on reducing the mean particle diameters. In the case of ultrasound, the diameter of the probe had a statistically significant influence on D (0.1) and D (3.2). Comparing different intensities of the ultrasonic probe, 7 and 10 mm, 10 mm had a slightly greater influence on fat globule distribution of goat milk (Table 1 and 2). Influence on sphericity of smaller

Table 1 – *Influence of US process parameters on particle diameters D (3,2); D (0,1)*

Process parameters			Particle diameter [μm]	
A[%]	*t*[min]	*d*[mm]	D (3,2)	D (0,1)
60	3	7	0.351	0.116
60	6	7	0.320	0.111
60	9	7	0.337	0.117
60	3	10	0.343	0.118
60	6	10	0.274	0.101
60	9	10	0.294	0.111
100	3	7	0.359	0.12
100	6	7	0.29	0.104
100	9	7	0.349	0.122
100	3	10	0.337	0.117
100	6	10	0.225	0.098
100	9	10	0.202	0.097
0	0	0	0.516	0.158

Table 2 – *ANOVA: Influence of ultrasound (US) and high hydrostatic pressure (HHP) process parameters on mean particle diameter [μm] of goat milk expressed over p – value*[*]

Process parameters		d(0,1)	d(0,5)	d(0,9)	d(3,2)	d(4,3)
HHP	*p*[MPa]	**0.010380**	**0.001461**	**0.001618**	**0.006959**	**0.001685**
	t[min]	**0.033358**	**0.009550**	**0.020460**	**0.027117**	**0.015668**
US	*A*[%]	0.334781	0.976110	0.768850	0.287436	0.770122
	t[min]	0.228341	0.168762	0.467840	0.101641	0.297538
	d[mm]	**0.015505**	0.362706	0.652941	**0.016547**	0.496661

[*]($p < 0.05$, statistically significant)

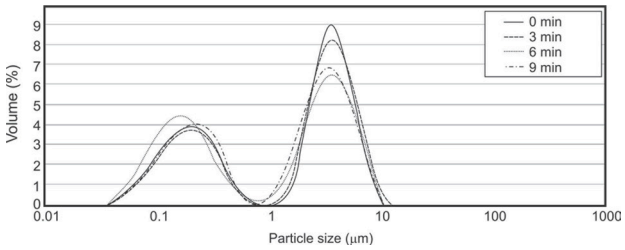


Fig. 4 – *Influence of US on Log – N distribution of goat milk (A = 100 %, d (probe) = 7 mm)*

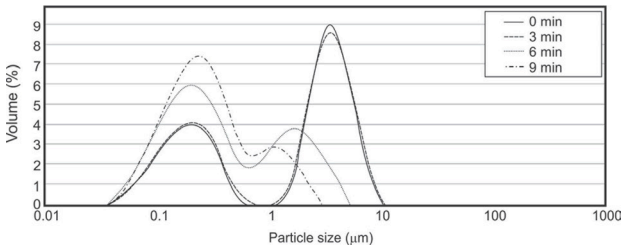


Fig. 5 – *Influence of US on Log – N distribution of goat milk (A = 100 %, d (probe) =10 mm)*

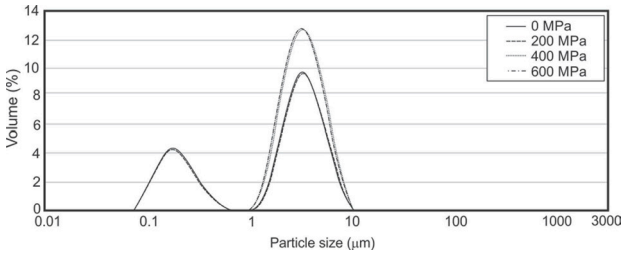


Fig. 6 – *Influence of HHP on Log – N distribution on goat milk after 3 minutes of treatment*

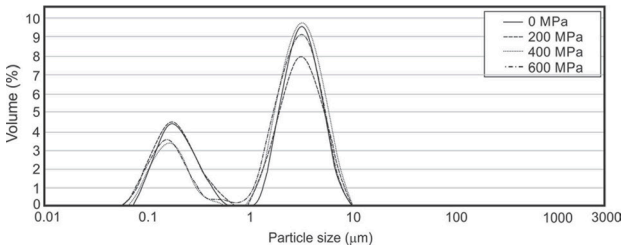


Fig. 7 – *Influence of HHP on Log – N distribution on goat milk after 9 minutes of treatment*

fat globules is also confirmed by statistically significant influence of the probe on D (3.2). In the absence of statistical significance, by increasing the amplitude to maximum 100 W and treatment time from 3 to 9 minutes, frequency of smaller fat globules had increased (Figs. 4 and 5). The meaning of value D (3.2) is specially expressed in characterization of fat globules of goat milk because of its natural homogeneity. Optimal globule size distribution is in the interval between 0.3 and 4 μm and the suitable value of variance between 0.2 and 0.6 is obtained using ultrasound.

There are no significant changes in distribution at 200 – 600 MPa at 3 minutes of pressurization (Fig. 6). Considering the given *β – coefficient* (Table 3), high pressure parameters indicate a two-fold influence on mean particle diameters. Observed diameters increase proportionally with increase of

Table 3 – *ANOVA: Influence of ultrasound (US) and high hydrostatic pressure (HHP) process parameters on mean particle diameter [μm] of goat milk expressed over β – standardized coefficient**

Process parameters		d(0,1)	d(0,5)	d(0,9)	d(3,2)	d(4,3)
HHP	*p*[MPa]	3.471831	5.061371	4.970635	3.772665	4.934788
	t[min]	–2.64135	–3.53369	–2.98187	–2.78463	–3.17182
US	*A*[%]	*	*	*	*	*
	t[min]	*	*	*	*	*
	d[mm]	–2.97779	*	*	–2.93774	*

[*] *– statistically not significant (p>0.05) – based on Table 2*

pressure, while time of treatment has opposite effect. Uniformity of *β – standardized coefficient*, pressures (*β = 3.47 – 5.06*) and time (*β = – 2.64 – (–3.53)*) appear to equally influence all mean particle diameters values. Therefore, optimal homogeneity of goat milk is achieved at 200 MPa and 9 minutes of treatment. This can be seen on both bimodality sides of Log – N distribution (Fig. 7). Obviously, resulting in increasing of the relative frequency of fat globules with diameter in interval from 0.1 μm up to 1 μm, and decreasing the frequency curve in range from 1 – 10 μm. The fat globules produced by high hydrostatic pressures influence the formation of clusters of fat globules and casein micelles. Negative *β – coefficient* (Table 3) indicates a decreasing influence of time on mean particle diameter value. In accordance with the given results, the optimally controlled process is under the lowest value of pressure used, with longer treatment time. The positive effect of high pressure process parameters in relation to mean particle diameters is shown in Fig. 8.

Energy density calculated over measured power of ultrasonic processor indicates similar energy consumption during treatment at both probes. Juliano *et al*. (2014) has shown the influence of energy on volatile compounds of various types of milk. At values lower than 271 J mL^{-1}, there are no volatile compounds from lipid oxidation in goat milk. Maximum amplitude 100 W and 9 minutes of treatment with 7 mm probe indicate 291.6 J mL^{-1} (Table 4). Received quantity of emitted energy is achieved at the last seconds of sonication. Chemical degradation of milk can be improved by controlling the sonication time and temperature of milk. After the sonication, temperature of sample did not exceed 32 °C. Final temperature of sample depends of chemical composition of milk, especially fat content.[26]

Table 4 – *Energy consumption (US) – expressed over Energy density [J mL^{-1}]*

Probe diameter [mm]	Energy density [J mL^{-1}]			
	A[%]/*t*[min]	3	6	9
7 mm	60	51.8	103.68	155.52
	100	97.2	194.4	291.6
10 mm	60	42.48	84.96	127.44
	100	73.44	146.88	220.32

In the case of HHP energy consumption, it is important to notice different distribution of energy during the treatment in comparison with ultrasound. Two high pressure pumps and two intensifiers are the main consumers of energy during the pressurising phase, as the pressurising part of the cycle is set at 10 MPa per second. Twenty seconds of pumping is needed for achieving 200 MPa. The hold part of the cycle uses a minimum amount of energy (mainly for electronics such as PLC's and computer). The depressurizing part of the cycle uses 4 mechanical valves and 4 pumps, which also consumes a negligible amount of energy compared to the pressurising part. The power drawn in a complete cycle

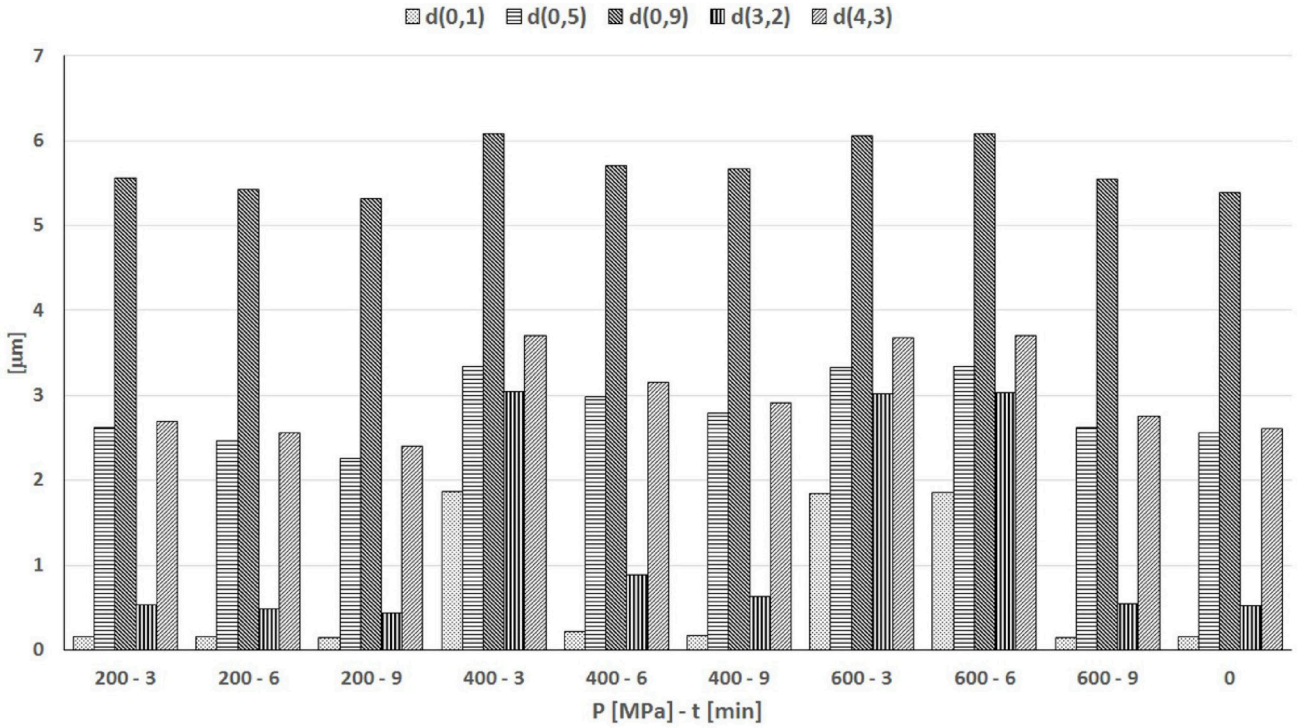


Fig. 8 – *Influence of HHP process parameters on mean particle diameters [μm] of goat milk*

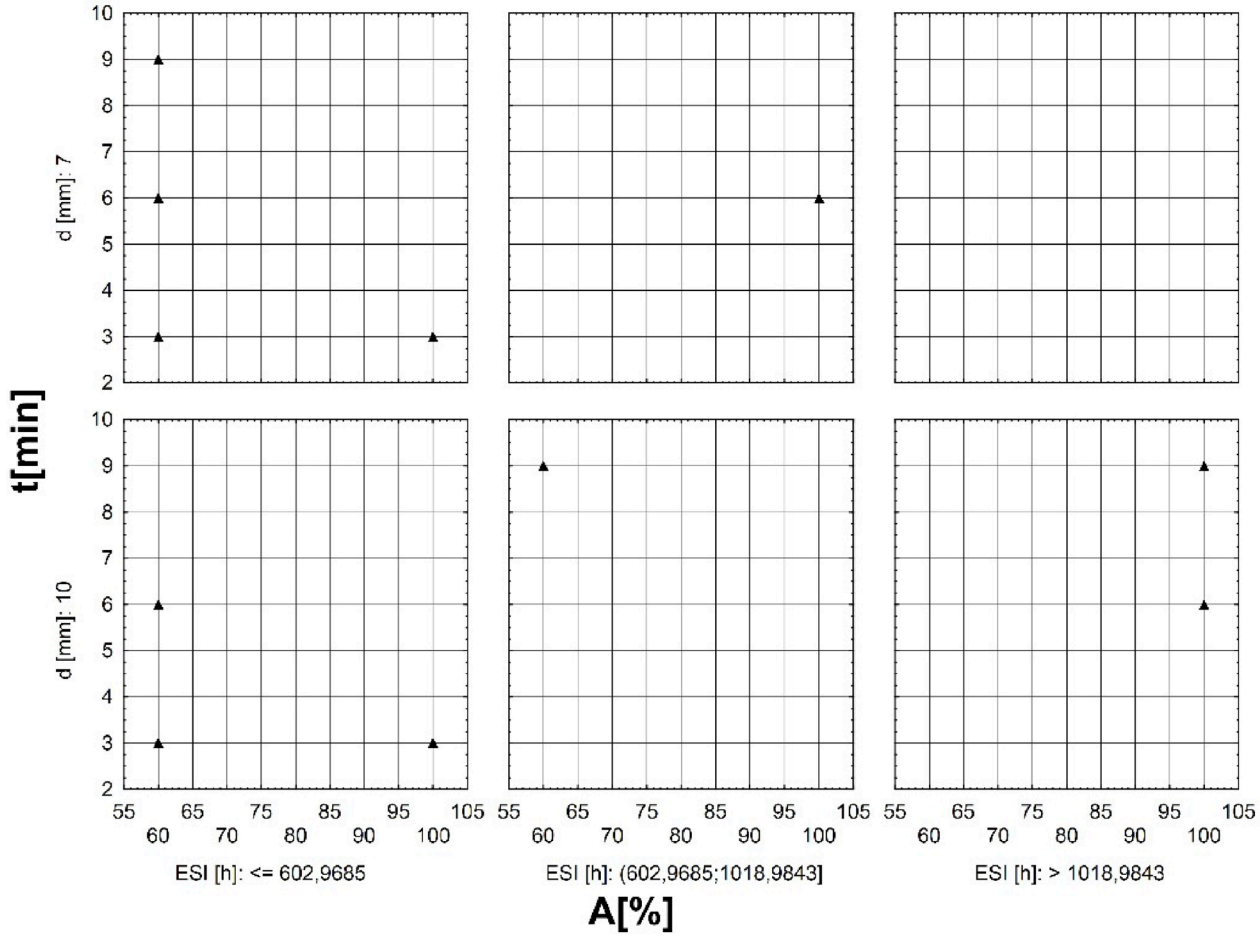


Fig. 9 – *Influence of US process parameters on ESI of goat milk*

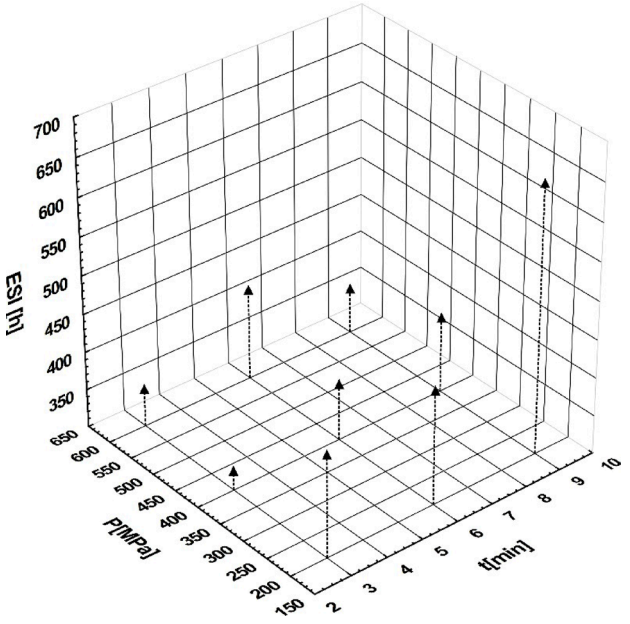


Fig. 10 – *Influence of HHP process parameters on ESI of goat milk*

(pressurising-hold-depressurizing) is about 40 W for pressurising of 2 L at 200 MPa (7200 Wh of energy).[28]

Stability of US and HHP homogenised milk can be expressed over ESI. A large number of US emulsions with ESI interval between 0 – 600 hours were obtained by 60 % amplitude, 3 and 6 minutes of treatment by both probes (Fig. 9). Emulsions obtained with HHP have intervals between 300 – 620 hours. The highest value of ESI were obtained at 200 MPa and 3, 6, and 9 minutes of treatment (Fig. 10). It is very important to emphasize that all obtained emulsions obtained by represented process parameters are significantly acceptable and stable.

Conclusions

The influence of high intensity ultrasound and high hydrostatic pressure processing on fat globule distribution size of goat milk is investigated. US and HHP homogenization of the milk lead to an increased total area of fat globules. The influence of imploding cavitation bubbles has shown better capability of destructing milk fat globules membrane, which consequently leads to improved homogeneity of such produced emulsion. As previously mentioned, in the case of high hydrostatic pressure, a complex of clusters of fat globules and milk pro-

teins are present. Milk proteins, especially casein, adsorb on the surface of the fat globule membrane. In that form, they function as a natural emulsifying agent. In addition, there is no need for the addition of an active surface material in milk. In inhomogeneous and homogeneous milk are present fat globules from 0.1 (and smaller) to 0.3 μm. High resistance of such small fat globules is achieved using both represented non-thermal homogenization techniques. After homogenization, with both non-thermal techniques, the emulsions were very stable and consistent over a long period.

List of symbols:

$\overline{d}$ – mean value, [μm]
μ – dynamic viscosity, [Pa s]
A_{500nm} – absorbance
c_v – propagation velocity of ultrasonic wave, [m s^{-1}]
$D\ (0.1)$ – 10 % of the volume distribution is below observed diameter, [μm]
$D\ (0.5)$ – median diameter, 50 %, [μm]
$D\ (0.9)$ – 90 % of the volume distribution is below observed diameter, [μm]
$D\ (3.2)$ – surface area mean – Sauter mean diameter, [μm]
$D\ (4.3)$ – volume moment mean – De Brouckere mean diameter, [μm]
d_c – diameter of cuvette [m]
d_i, d – diameter of fat globules, [μm]
ESI – Emulsion Stability Index [h]
n – number of fat globules
s_p – surface area of one particle, [m^2]
T – optical density
V_p – volume of one particle, [m^3]
x_p – equivalent diameter of one particle, [m]
α – attenuation coefficient, [Neper m^{-1}]
ΔT – optical density variation over the 24 hours.
Δt – time interval [24 h]
ρ – density, [kg m^{-3}]
σ – variance
Φ – sphericity
ω – nominal frequency of selected probe, [Hz]

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