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Full Length Research Paper

Influence of high intensity ultrasound with different probe diameter on the degree of homogenization (variance) and physical properties of cow milk

Bosiljkov, T.*, Tripalo, B., Brnčić, M., Ježek, D., Karlović, S. and Jagušt, I.

Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia.

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The main goal of this research is to analyze the influence of ultrasonic probe diameters (7 and 10 mm) of high-intensity ultrasound with constant frequency (30 kHz) on the degree of homogenization (variance) of cow milk. Influence of different probe diameters on the physical properties of cow milk was also tested. Changes in temperature, pH and density were measured under the following operational conditions of the ultrasonic device: amplitude, A = 20, 60,100%; and applied cycle, c = 0.6, 0.8, 1, with various treatment duration, t = 2, 6, 10, 15 min. Obtained results are processed in the "Statistica 8" software. Microscopic images of fat globules were edited in Image J software, while size of fat globules was represented with Log-normal distribution. Statistical analysis was conducted and influence of probe diameters on physical properties was expressed over p-value ($p < 0.05$) and *β*-standardized coefficient analyses of variance (ANOVA). Applications of different probe diameter have significant influence on all physical properties and variance. With increase of the amplitude and time, significant influence on variance (degree of homogenization) is observed.

Key words: Ultrasound, homogenization, cow milk, physical properties.

INTRODUCTION

Today in food technology and industry, new innovative processes are arising. All these technologies are based on known techniques, but still not enough to be applied to food stuffs manufacturing. Usually, non-thermal technologies such as high hydrostatic pressure (Patterson et al., 2006; Alegre et al., 2010), oscillating magnetic fields (San Martin et al., 2001), pulsed light (Hierro et al., 2009; Marquenie et al., 2003), cold plasma (Nardi et al., 2009; Basaran et al., 2008), pulsed electric fields (Huang and Wang 2009; Gerlach et al., 2008) and power or hi-intensity ultrasound (Dolatowski et al., 2007; Mason et al.,1996) find their place in processing of foods.

Implementation of high intensity ultrasound in food technology and biotechnology became one of the most progressive technologies that are used in many technological processes in food industry. The most represented are drying, mixing, homogenization, extraction, crystallization and many more (Bosiljkov et al., 2009; Herceg et al., 2009; Brnčić et al., 2009). High intensity ultrasound (intensity above 1 W/cm^2, usually in the ranges of 10 and 1000 W/cm^2 and frequencies of 20 to 100 kHz) is suitable to change physico-chemical properties of treated materials. Past researches showed that intensities above 1 W/cm^2 have significant effect on emulsion formations, therefore reducing the size of oil droplet or fat globules in milk (Mc Clements, 2005; Wu et al., 2001; Ertugay et al., 2004).

Stability and physico-chemical properties of food emulsion usually depend on the method used in creating it. The most acceptable is application of different types of homogenizer like a high speed blender, high pressure homogenizer, ultrasonic jet homogenizer, membrane processing and ultrasonic homogenization influenced by high intensities (above 1 W/cm^2) of ultrasonic waves which are defined by diameter of the ultrasonic probe and nominal power of ultrasonic processor (Mc Clements, 2005). Emulsion stability considers increasing the stability of fat globule on sizes between 1 and 3 µm. In that range, globules cannot get to the top of the milk after storage

*Corresponding author. E-mail: tbosilj@pbf.hr. Tel: +385 1 4605-224. Fax: +385 1 4605-200.

and during consumption (Bosiljkov et al., 2009). Using a high intensity ultrasound is necessary in order to achieve a cavitations effect on treated milk. This effect depends on the frequency, amplitude and diameter of ultrasonic probe which is used. Implosion of cavitations gas bubbles produces high intensity shock waves which directly decrease the fat globules (Suslick, 1988; De Castro and Capote, 2007; Chucheval and Chow, 2008). The aim of this work is to show the influence of different ultrasonic process parameters with different types of ultrasonic probes on degree of homogenization of cow milk expressed over variance and changes observed on physical properties.

MATERIALS AND METODS

Sample

Inhomogeneous cow milk has the following chemical compositions: protein content, 3, 6% (N × 6, 25); total sugar, 4, 2%; fat content, 4, 2%; minerals, Ca-1104,13 mg/kg, P - 0, 071%. Physical properties of inhomogeneous cow milk are: pH-value, 6,462; density (ρ), 1, 0311 g / cm^3; specific heat capacity, c_p = 3,751 J / kg°C. Degree of homogenizations of inhomogeneous cow milk expressed over variance is (V(x)) = 2.

Ultrasound treatment

Inhomogeneous cow milk was homogenized with ultrasonic processor "UP 100 H" produced by "Dr Hielscher" Company (Teltow, Germany) with constant frequency of 30 kHz, using cylindrical probes with different diameter: S7 (d_{S7} = 7 mm) and S10 (d_{S10} = 10 mm). Ultrasonic treatments were performed using the following: amplitude, A = 20, 60, 100%; time of treatment, t = 2, 6, 10, 15 min; and applied cycles, c = 0, 6; 0, 8; 1. Volume of the sample was 150 ml with immersion depth of the probe as 3 cm.

Determination of intensities

Sonication was conducted and intensities were determined using ultrasonic processor "UP 100 H", with constant frequency of 30 kHz using cylindrical probes: S7 (d = 7 mm) and S10 (d = 10 mm). Measurement conditions (amplitude and time) were identical, such as ultrasonic sonication with full applied cycle (c = 1). Inside the calorimetric system, measuring of changes in temperature provides possibilities to calculate output acoustic power. Differences in temperature (ΔT) were measured every 10 s using thermometer with sensors "DS1820" and microcontroller "ATMEL and MAX232". Changes in acoustic power (ΔU) can be described with equation 1:

$$\Delta U = \Delta Q = \sum_{i=1}^{N} m_i \times c_i \times \Delta T = c_{tot} \times \Delta T \qquad (1)$$

Where m_i, mass of the individually part of the system; ΔT, total changes of temperature in system; c_i, specific heat capacity of individual part of the system; N, number of elements; c_{tot} = total heat capacity.

Standard deviation (ΔP_a) of output acoustic power can be given with the following equation 2:

$$\Delta P_a = c_{tot} \times \Delta A \qquad (2)$$

Where ΔA, differences between two temperature sensors that are time dependent.

Acoustic intensity (I_a) is described with equation 3:

$$I_a = \frac{\Delta P_a}{S} = \frac{\Delta P_a}{r^2 \pi} \qquad (3)$$

Where S, surface area of the probe.

Microscoping

After the ultrasonic treatment, samples were monitored and analyzed under the light microscope (XSP-8F-0403) with magnification of × 1000. Pictures of fat globules were recorded with digital camera (Olympus E- 520 KIT) and analyzed with "Image J" software.

Measurement of temperature, pH- value and density

During the ultrasonic treatments, temperature was measured every 30 s using IR-measurer (Rytek) and ray was directed on observed sample with direct reading on display. Starting temperature of inhomogeneous milk was 20°C. Similarly, measurements of pH-value were performed at 20°C using a calomel electrode of pH - meter (WTW 330 i / SET). Work temperature of density meter (Mettler Toledo DE 40) was set on 20°C. After the correction of the sample temperature, density of treated milk was displayed.

Statistical analysis

Statistical analysis was performed using Statistica 8 software. Total influence of different probe diameter on physical properties is expressed over p-values ($p < 0.05$) and β-standardized coefficient, with numerical value (positive or negative) indicating increase or decrease in observed physical properties. Statistical significant differences between each probe are expressed over p-values (Post-hoc analyses: Tukey test). Significant changes are given by a comparison of physical properties of inhomogeneous milk.

For most quality presentation and quantitatively description of degree of homogenization (variance), characterization of fat globule size distribution was produced. Characterization model using raw data analyzed by Image J software was represented with Log-normal distribution model expressed with equation 4:

$$f(d) = \frac{1}{\sqrt{2\pi} d \ln\sigma} \exp\left(-\left[\frac{\ln d - \ln \bar{d}}{\sqrt{2}\ln\sigma}\right]^2\right) \qquad (4)$$

Where d, diameter of fat globules; $\bar{d}$, mean value; σ, variance (Gregory, 2006).

RESULTS AND DISCUSSION

Physical properties of ultrasonically treated milk are presented in Figures 1 to 3, while the influence on changes

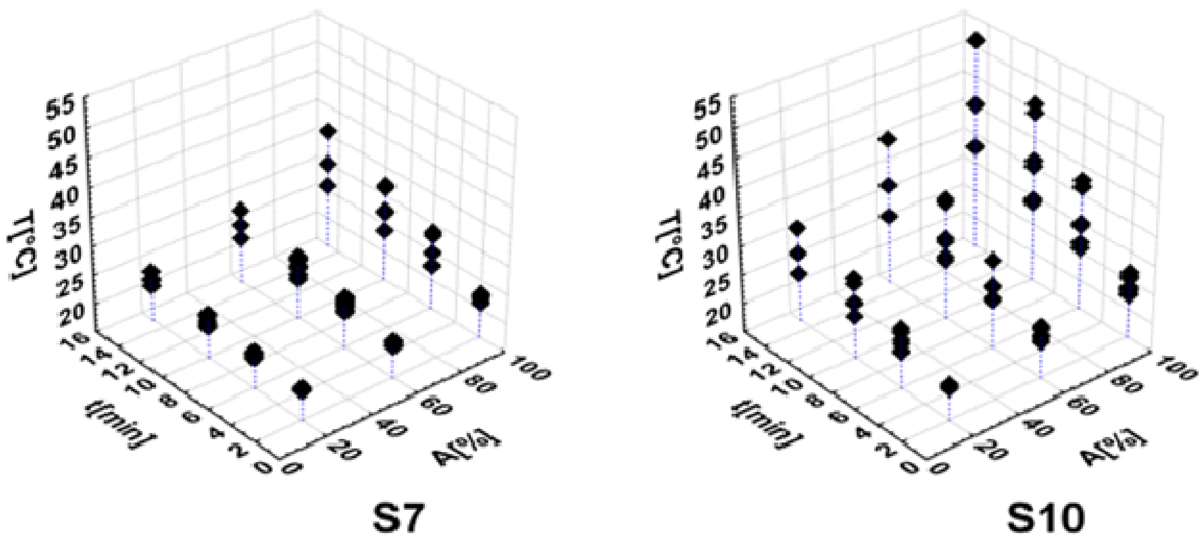

Figure 1. Influence of amplitude, time of treatment and applied cycles on temperature (T) of cow milk categorized by different diameter of the probe (S7 and S10).

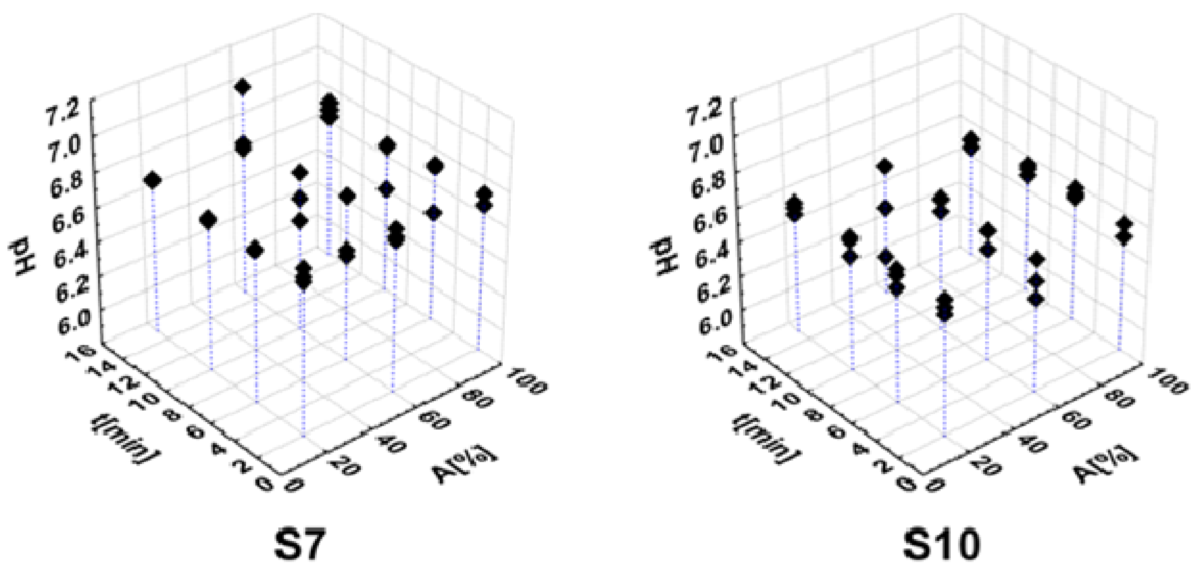

Figure 2. Influence of amplitude, time of treatment and applied cycles on pH-value of cow milk categorized by different diameter of the probe (S7 and S10).

variance (degree of homogenization) is shown in Figure 4. Total influence of both probes expressed over *p*-value and β-coefficient on all physical properties of cow milk and variance (degree of homogenization) is represented with ANOVA in Table 1. Influence of probes shows significant influence on all observed physical properties ($p < 0.05$). Positive value of β-coefficient indicates increase in temperature and density of cow milk, while negative value of β-coefficient indicates decrease in pH-value and variance (degree of homogenization).

Changes in pH-value depend on physico-chemical properties of milk and are mostly invariable after sonication. Increase in density can be described as the phenomenon that is based on the differences between plasma and fat globules density, therefore, they have tendency to increase their volume. After ultrasonic homogenization, lower density of fat milk indicates a formation of protein layer covering the surface of fat globules. In this case, it is possible to increase the density of fat globules (Mc Clements, 2005).

Post-hoc analyses (ANOVA-Tukey test) showed individual significant influence ($p < 0.05$) of each probe separately in Table 2. Intensities for each probe (S7, S10), categorized by amplitude are given in Table 3.

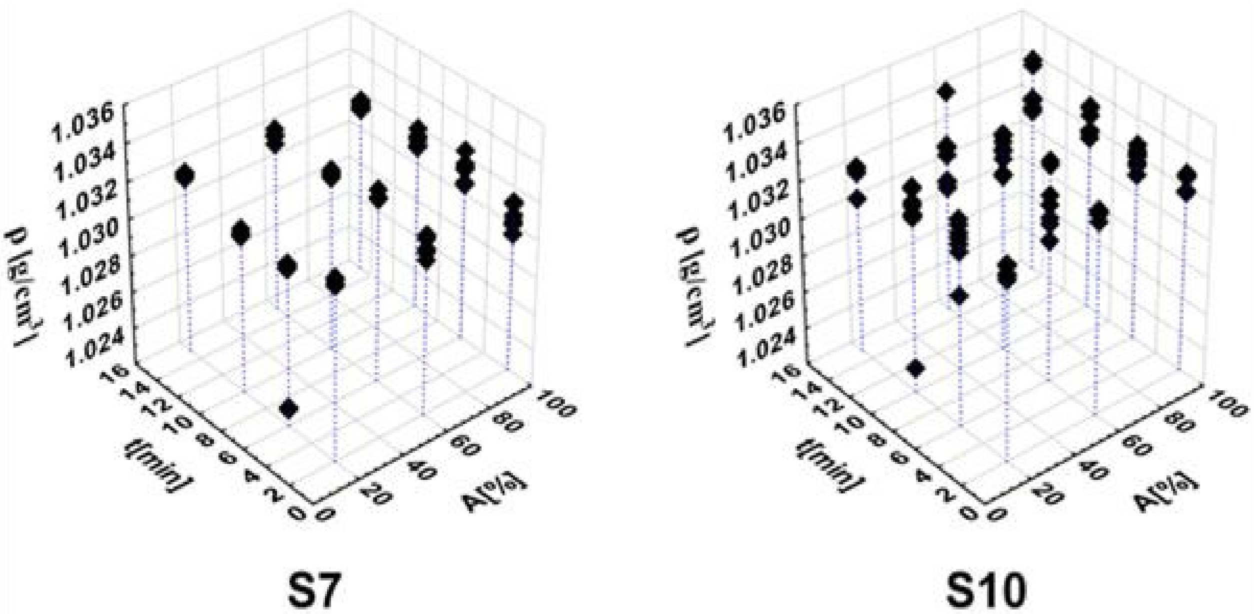

Figure 3. Influence of amplitude, time of treatment and applied cycles on density (ρ) of cow milk categorized by different diameter of the probe (S7 and S10).

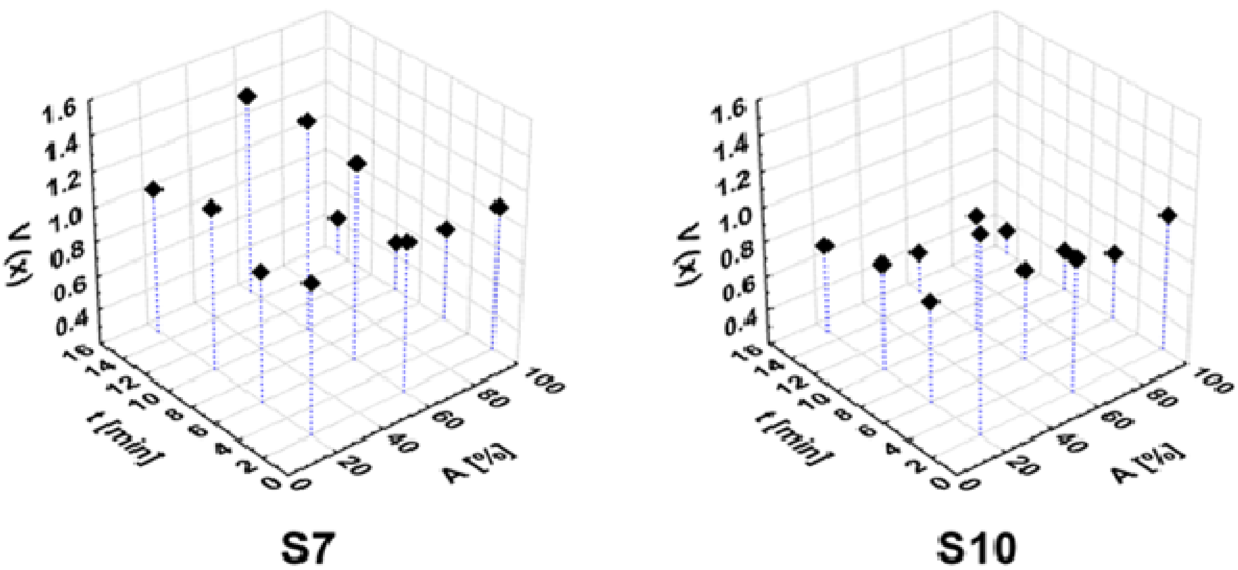

Figure 4. Influence of amplitude, time of treatment and applied cycles on variance (*V(x)*) (degree of homogenization) of cow milk categorized by different diameter of probe (S7 and S10).

Influence of the probe, S10 (lower maximum intensities 104.59 W / cm^2) showed significant influence on changes of temperature and density of milk, while probe S7 (higher maximum intensities 134.95 W / cm^2) showed no effect. Contrary effect was noticed using probe S10 (lower intensities) on increasing temperature. Reason for high rise of temperature is the influence of the probe on higher observed volume. Decrease in pH-value significantly influenced probe S7, while probe S10 has no influence.

Both probes significantly influenced decrease in variance (degree of homogenization) by emphasizing on probe S10 whose p – value is more significant ($p_{S10} < p_{S7}$). Influence of different probe diameter has no effect on parameters of Log-normal distribution like median, mean and maximum diameter of fat globules. Maximum

Table 1. ANOVA: Total influence of probe diameters on physical properties of milk and variance *V(x)* (degree of homogenization) expressed over *p*-value ($p < 0.05$) and *β*-standardized coefficient.

Variable	*p*-value	β–Standardized coefficient
Temperature (°C)	0.000001	5.058395
pH - value	0.000000	- 8.74293
Density (g/cm^3)	0.000119	3.920073
Variance V(x)	0.017433	- 2.5918

Statistically significant when ($p < 0.05$); ANOVA, analysis of variance.

Table 2. ANOVA: Post-hoc analyses Tukey test, individual influence of different probe diameters (S7 and S10) on physical properties of milk and Variance *V(x)* (degree of homogenization) expressed over *p*-value ($p < 0.05$).

Variable	S7 *p*-value	S10 *p*-value
Temperature (°C)	0.108351	0.000025
pH - value	0.003754	0.850644
Density (g/cm^3)	0.444252	0.000036
Variance V(x)	0.001359	0.000266

Statistically significant when ($p < 0.05$); ANOVA, analysis of variance.

Table 3. Acoustic intensities (I_a) for each probe (*d*) at different amplitudes (*A*).

Mark / d (probe) (mm)	A (%)	I_a (W/cm^2)
S7 / 7	20	35.78
S7 / 7	60	74.00
S7 / 7	100	134.95
S10 / 10	20	28.44
S10 / 10	60	58.46
S10 / 10	100	104.59

difference in maximum diameters of fat globules was observed between inhomogeneous cow milk (d_{max} = 8.9 µm) and samples at maximum intensities and time of treatment for probe S7 (Δd_{max} = 4.7 µm) and probe S10 (Δd_{max} = 3.7 µm).

According to Wu et al. (2001), decrease in fat globules is observed only by microscopic observation, and longer exposure time leads to better homogenization. Figure 5 showed microscopic pictures of fat globules with corresponding Log-normal distribution. In spite of the variation of intensity ultrasonic waves, homogenization efficiency can be improved by sonication for a longer time. Longer time of treatment significantly ($p < 0.05$) influences better distribution (decreasing of variance) at both applied probes (Figure 5). Furthermore, Ertugay et al. (2004) and Reiner et al. (2009) noticed that, the highly applied amplitude of ultrasonic device leads to formation of smaller fat globules with diameter smaller than 1 µm. The 90% of fat globules were in the ranges of 0.5 and 0.6 µm. Amplitude and time of treatment significantly influenced ($p < 0.05$) decrease in median, mean and maximum fat globules. It is clear that, minimum time of treatment (2 and 6 min) had effect on minimizing production of clusters, which can induce instability of such produced emulsion because of increasing surface area between phases and decreasing interfacial tension (Mc Clements, 2005; Abismaïl et al., 2000).

Conclusion

Influence of different intensities of applied probes showed changes on all observed physical properties. Significant influence using probe S10 (lower maximum intensities) is noticed during observation of temperature and density. Probe S7 (higher maximum intensities) influenced pH-value. Both probes showed statistical influence on degree of homogenization (variance). Relative frequency of

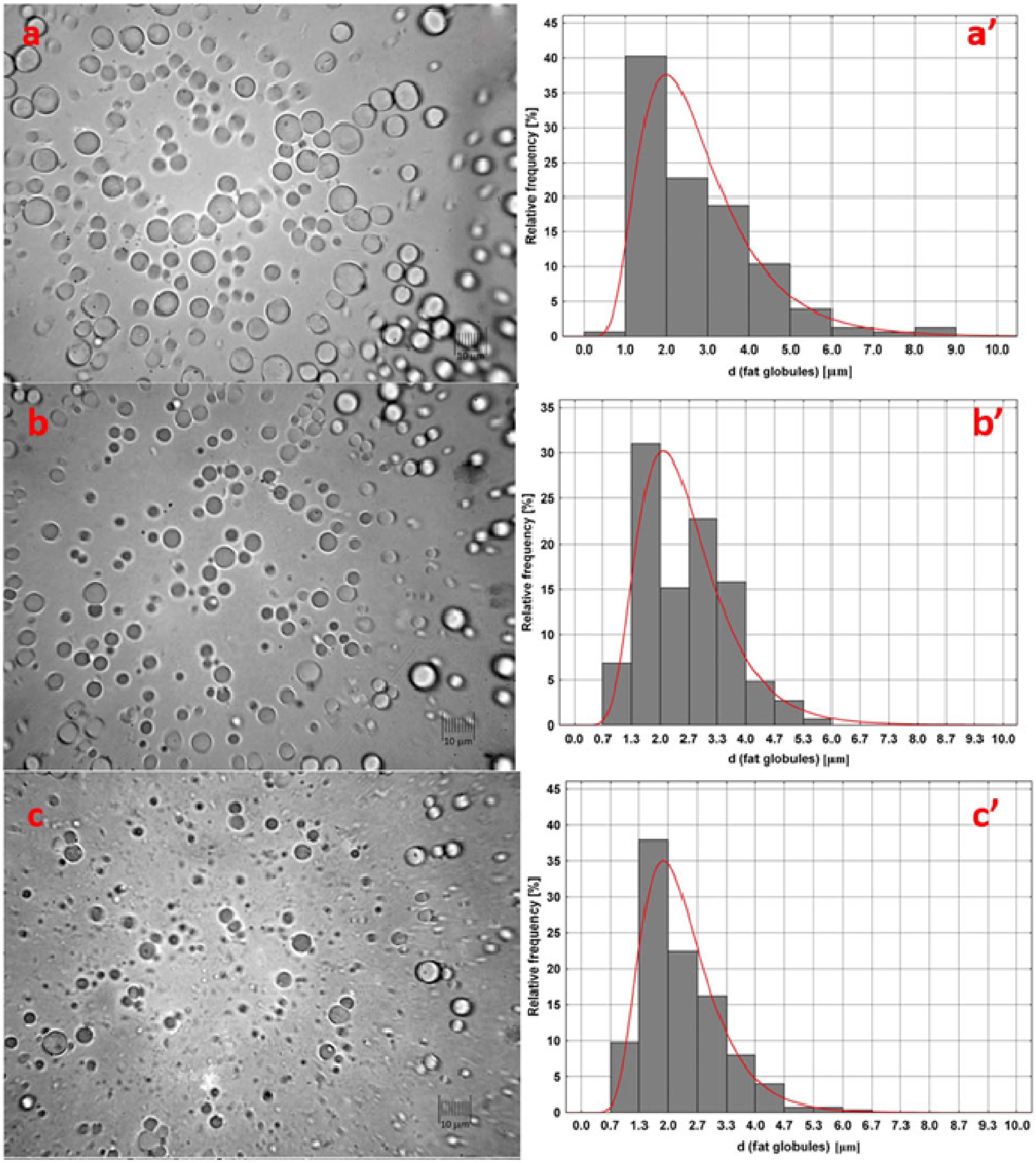

Figure 5. Microscopic pictures of fat globules before and after ultrasonic treatment with corresponding Log-normal distribution (a', b', c', d', e') by cycle (c = 1) and time (t = 10 min); **a**, inhomogeneous cow milk; **b**, A = 20 %, S7; **c**, A = 20 %, S10; **d**, A = 100 %, S7; **e**, A = 100 %, S10.

inhomogeneous cow milk was in the ranges of 0.7 and 8.9 µm. After sonication, relative frequency of observing fat globules for both probes (S7, S10) was in the ranges of 0.7 and 5.0 µm. Over 50% of all fat globules (relative frequency) are in the ranges of 0.7 and 3 µm. As such, this proves a successful homogenization and forms a

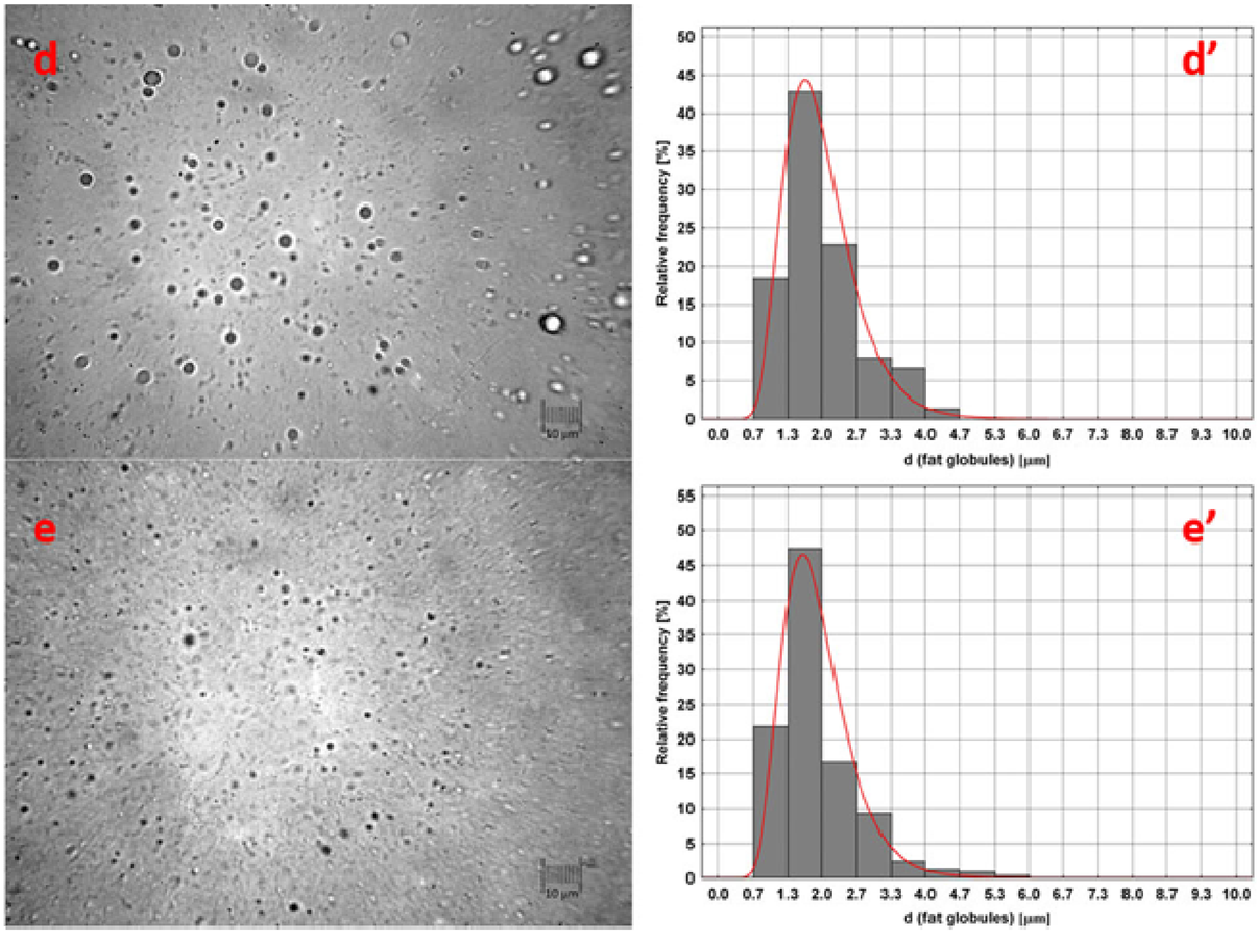

Figure 5. Contd.

very stable emulsion.

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