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Application of high intensity ultrasound treatment on *Enterobacteriae* count in milk

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Summary

Ultrasonication is a non-thermal method of food preservation that has the advantage of inactivating microbes in food without causing the common side-effects associated with conventional heat treatments, such as nutrient and flavour loss. In this work high intensity ultrasound was used to investigate inactivation *Enterobacteriae* count in raw milk. Raw milk with 4% of milk fat was treated with ultrasonic probe that was 12 mm in diameter and with 20 kHz frequency immerged in milk directly. For ultrasounds treatment, three parameters varied according to the statistical experimental design. Centre composite design was used to optimize and design experimental parameters: temperature (20, 40 and 60 °C), amplitude (120, 90 and 60 μm) and time (6, 9 and 12 minutes). All analyses were performed immediately after sonication and after 3 and 5 days of storage in refrigeration at 4 °C. The facts that substantially affect the inactivation of microorganisms using ultrasound are the amplitude of the ultrasonic waves, the exposure/contact time with the microorganisms, and the temperature of treatment. The achieved results indicate significant inactivation of microorganisms under longer period of treatments with ultrasonic probe particularly in combination with higher temperature and amplitude. Output optimal value of *Enterobacteriae* count has been defined by Statgraphics where lowest *Enterobacteriae* count (1.06151 log CFU mL^{-1}) was as follows for specific ultrasound parameters: amplitude of 120 μm, treatment time for 12 min and temperature of 60 °C.

Key words: High intensity ultrasound, milk, *Enterobacteriae* count, D-values

Introduction

Heat treatment is generally used to treat milk to control microbial growth. Heat processing is not effective against all microbes (Povey and Mason, 1998; Floros and Liang, 1994; Guerrero at al., 2001) associated with milk and may trigger unwanted reactions such as loss of flavour, nutrients and vitamins (Mason, 1990). To avoid the unwanted effects of heat, efforts are being made to find alternate methods of food preservation, based on new inactivation procedures (McClements, 1995). Ultrasonication is a non-thermal method of food processing that has the advantage of inactivating microorganisms in food without causing the common side-effects associated with conventional heat treatments. Food processing using ultrasound involves the transmission of energy at frequencies higher than 20 kHz. The low-intensity ultrasound which uses very small power levels (typically less than 1 Wcm^{-2}, with the frequency range of 5-10 MHz), causes no physical and chemical alterations in the properties of treated material. Because of that low power ultrasound can be used to measure the texture, composition, viscosity or concentration of food. In contrast, the high-intensity ultrasound which uses much higher power

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levels (typically in the range of 10-1000 Wcm^{-2}, with the frequency of 20-100 kHz), causes physical disruption of the material to which it is applied and promotes certain chemical reactions (Mason, 1998). Thereby high- intensity ultrasound is considered to be a potential unit operation of non-thermal processing of food whose possible effects including the disruption of microorganisms and enzymes, generation of dispersion and emulsion or promotion of certain chemical reactions (Floros and Liang, 1994; Mason, 1998; McClements, 1995). The mechanism of microbial killing is mainly due to thinning of cell membranes, localized heating and production of free radicals (Butz and Tauscher, 2002; Fellows, 2000; Piyasena et al., 2003; Cameron et al., 2008; Villamiel and de Jong, 2000). During the sonication process, longitudinal waves are created when a sonic wave meets a liquid medium, thereby creating regions of alternating compression and expansion (Sala et al., 1995). These regions of pressure change cause cavitation to occur, and gas bubbles are formed in the medium. These bubbles have a larger surface area during the expansion cycle, which increases the diffusion of gas, causing the bubble to expand. A point is reached where the ultrasonic energy provided is not sufficient to retain the vapour phase in the bubble; therefore, rapid condensation occurs. The condensed molecules collide violently, creating shock waves. These shock waves create regions of very high temperature and pressure, reaching up to 5500 °C and 50,000 kPa. The pressure changes resulting from these implosions are the main bactericidal effect in ultrasound. The hot zones can kill some bacteria, but they are very localized and do not affect a large enough area.

Microorganisms are relatively resistant to the effects, thus extended periods of ultrasonication would be required to render a product safe. If ultrasound were to be used in any practical application, it would most likely have to be used in conjunction with pressure treatment (manosonication), heat treatment (thermosonication) or both (manothermosonication). The enhanced mechanical disruption of cells is the reason for the enhanced killing when ultrasound is combined with heat or pressure. Pioneering work in this area was done by Ordonez et al. (1984) using ultrasound of 20 kHz and 160 W combined with temperatures ranging from 5 to 62 °C. The combination of heat and ultrasound was much more efficient with respect to treatment time and energy consumption compared to either treatment individually (Ordonez et al., 1984).

The objective of this work was to investigate the effect of high intensity ultrasound, using ultrasound frequency of 20 kHz, and changing various conditions (treatment time, amplitude and temperature) on *Enterobacteriae* count in milk. Bacterial count has been measured immediately after ultrasonic treatment, and after 3 and 5 days of storage.

Materials and methods

Milk samples

Raw cow's milk with 4% of milk fat was kept in refrigeration at 4 °C until used. The initial microbial load of *Enterobacteriae*, pH and titratable acidity (SH) was tested for all milk samples - raw milk (R), pasteurized milk (P), sonicated (US) and thermosonicated (TS). Pasteurized milk (P) was treated in plate heat exchanger in Natura agro d.o.o at temperature 85 °C, 15 sec. All analyses were performed immediately after sonication and after 3 and 5 days of storage in refrigeration at +4 °C.

Microbiological analysis

Serial dilutions were made in Peptone water (0.1 %) with samples taken from raw milk, sonicated and thermo-sonicated milk to evaluate the microbial load.

Enumeration of *Enterobacteriae* - from proprieties decimal dilution 1 ml of sample has to be inoculated deeply in VRBG media - Violet red bile glucose agar in Petri dish (optimal agar temperature 45 °C). The dishes are left to solidify agar and incubated at 37±1 °C/24 hours. The colonies are counted and result calculated, CFU mL^{-1} of sample (ISO 7402:1999).

Microbiological analyses were performed after ultrasonic or termoultrasonic treatment and after 3 and 5 days of storage in refrigeration at 4 °C. All microbiological analyses were conducted at least in triplicate for each experiment.

Ultrasound treatments

Raw milk (200 mL) was placed in a double-walled vessel (200 mL), which served as the treat-

ment chamber. An ultrasonic processor (S-4000, Misonix Sonicators, Newtown, Connecticut, USA), set at 600 W, 20 kHz, 12-260 μm with a 12 mm diameter probe, was introduced into the vessel. Ultrasonication were carried out with 60, 90 and 120 μm amplitude. The raw milk samples were treated by ultrasonic 6, 9 and 12 minutes and transferred to tubes with peptone water to perform microbiological analysis. In the case of thermosonication before ultrasonic treatment the samples were heated at 20, 40 and 60 °C. Overheating of the samples was prevented by water cooling of the treatment chamber. Each experiment was conducted at least in triplicate. For this study, 16 samples were ultrasonically treated (Table 1).

Determination of acoustic power and efficacy of ultrasonic treatments in terms of eliminating microbes

The most widely accepted method for determining the power from an acoustic horn into an aqueous solution is the calorimetric technique described by Margulis and Maltsev (2003). This method involves taking a known volume of water and applying ultrasound (for ca. 3 min) while monitoring the change in temperature with time for various ultrasonic amplitudes. The ultrasonic power can be readily determined from the following equation:

$$P = m \times C_p \times \frac{\partial T}{\partial t} \quad (1)$$

where P is the ultrasonic power, m is the mass of the solvent used, C_{sys} is the specific heat capacity of the system, which is described by

$$C_{sys} = C_g M_g + C_w M_w \quad (2)$$

where C_g is the specific heat capacity of the container used (in this case glass) and M_g is the mass of the container the ultrasonic power can be deduced. This simple equation has been widely used throughout the sonochemistry literature.

The efficacy of ultrasonic treatments in terms of eliminating microbes was measured by their decimal reduction time (D). D value was calculated as the time (min) required to reduce the number of viable cells by one log cycle or to kill 90 % of popula-

Table 1. Treatment time, amplitude, temperature, intensity and added energy during ultrasound treatments

Samples	Amplitude (μm)	Treatment time (minute)	Temperature (°C)	Intensity (W cm^{-2})	Energy (J)
R	-	-	-	-	-
A1	60	12	20	53.98	58.548
A2	90	6	40	29.84	15.527
A3	120	6	20	27.84	21.521
A4	120	6	60	24.97	19.145
A5	90	9	60	50.08	38.136
A6	60	6	60	26.06	13.436
A7	120	12	60	54.59	60.125
A8	90	9	40	49.85	36.836
A9	60	9	40	41.13	28.392
A10	90	12	40	55.13	62.873
A11	120	12	20	57.49	64.348
A12	90	9	40	49.85	36.836
A13	60	6	20	24.99	12.763
A14	90	9	20	49,16	37.011
A15	120	9	40	52.16	43.084
A16	60	12	60	52.26	64.423

tion at a given temperature, time of ultrasonic treatment and sonic wave amplitude. D-values were calculated from the slope of the regression line plotted with the counts (CFU mL^{-1}) of the straight portion of the survival curve. In this study, the D-value at 20 kHz was abbreviated as D_{US}.

$$\log \frac{N_1}{N_0} = -\frac{t}{D_{us}} \qquad (3)$$

N_0 - number of total *Enterobacteriae* before ultrasound treatment, N_1 - number of *Enterobacteriae* after ultrasound treatment at time t, D_{us} - decimal reduction time (min)

Experimental methodology

Experimental designs such as Central Composite Designs (CCD) are useful for response surface methodology (RSM) because they do not require an excessive number of experimental runs. Response surface methodology (RSM), the statistical method, uses quantitative data from appropriate experiments to determine and simultaneously solve multivariate equations (Montgomery, 2001; Myers and Montgomery, 2002). It is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors, and analyzing optimum conditions of factors for desirable responses.

A general factorial design (STATGRAPHICS Centurion, StatPoint technologies, Inc, VA 20186, USA) consisting of 16 experimental trials have been designed and chosen to obtain general observation of ultrasound treatment of amount *Enterobacteriae* in milk. In order to determine influence of each factor on the count of *Enterobacteriae* central composite design (CCD), and face centred model was chosen. The ultrasound factors of amplitude (μm), temperature (°C), and treatment time (min) were studied. Analysis of variance (ANOVA) was carried out to determine any significant differences ($P<0.05$) among the applied treatments. The operating variables were considered at three levels namely, low (-1), central (0) and high (1). Accordingly, 16 experiments were conducted with experiments organized in a factorial design (including factorial points, axial points and centre point) and the remaining involving the replication of the central point to get good estimate of experimental error. Repetition experiments were carried out after other experiments followed by order of runs designed by program. Response (output) values were total *Enterobacteriae* count in log cfu ml^{-1}.

The designs were based on two-level full factorial design, which were augmented with centre and star points (Kuehl, 2000). The total number of experiments of the designs (N) can be calculated as follows,

$$N = N_i + N_o + N_j \qquad (4)$$

Where $N_i = 2^n$ is the number of experiments of the two level full factorial design, N_o is the number of centre points and $N_j = 2 \times n$, is the number of star points.

Response surface methodology

The experimental results were analyzed by response surface methodology (RSM) using the software STATGRAPHICS Centurion (StatPonit technologies, Inc, VA 20186, USA). Calculations were done at 95 % of confidence level. The ultrasound factors of amplitude - X_1 (μm), temperature - X_2 (°C) and treatment time - X_3 (min) were studied using RSM. In order to optimize the ultrasound treatment and investigate effects of above independent variables on the count of *Enterobacteriae*, a central-composite rotary design with the variables at three levels was used in the experiments (Table 1). Design matrix for the experiment and the regression model proposed for response was given below (Khuri and Cornell, 1996):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i<j}^{4} \beta_{ij} X_i X_j \qquad (5)$$

where β_0 is the value of the fixed response at the central point of the experiment which is the point (0, 0, 0); β_i, β_{ii} and β_{ij} are the linear, quadratic and cross-products coefficients, respectively. While demonstrating the significant effects 3-dimensional fitted surfaces were drawn (Lu, et al., 2008). The model was fitted by multiple linear regressions (MLR). The validity of the quadratic empirical model was tested with analysis of variance (ANOVA). The confidence level used was 95 %.

Results and discussion

In this work high intensity ultrasound was used to investigate inactivation of microorganisms in raw milk with 4 % milk fat. The amount of *Enterobacteriae* in milk after ultrasonic treatment were analyzed by response surface methodology (RSM) using the software Statgraphics Centurion. Calculations were done at 95 % of confidence level. In these different ultrasound treatments, the combination effect of amplitude as X_1, treatment time as X_2 and the temperature X_3 was studied using RSM. A general factorial design consisting of 16 experimental trials have been designed and chosen to obtain general observation of ultrasound treatment of amount *Enterobacteriae* in milk (Table 1).

The predicted model for count of *Enterobacteriae* in milk can be described by the polynom where E is count of *Enterobacteriae*, TT is treatment time (min); temperature is T (°C) and A is amplitude (μm):

$$E = 5{,}81204 + 0{,}00531236*A - 0{,}51721*TT - 0{,}0351543*T - 0{,}0000756705* A^2 + 0{,}000347222**A*TT + 0{,}0000520833* A*T + 0{,}017433* TT^2 + 0{,}000104167* TT*T + 0{,}000154741* T^2$$

All analyses were performed immediately after ultrasonic treatment and after 3 and 5 days of storage in refrigeration at 4 °C. The initial *Enterobacteriae* counts before milk processing were 4.08 log CFU mL^{-1}. According to national sanitary standards, the acceptable amount of *Enterobacteriae* count in the pasteurized milk is less than 2 log CFU mL^{-1} ($1*10^2$ CFU mL^{-1}) for pasteurized milk in bottles and packages (NN, 2001; Hillerton, 2004).

After milk treatment by ultrasound at 20 °C plate count was reduced to 2.98 (Sample A13) respectively 2.68 log CFU mL^{-1} (Sample A3) (Table 2). Significant difference in reduction of plate count *Enterobacteriae* is consequence different amplitudes of applied ultrasound. This research demonstrate that ultrasound treatment at ambient temperature

Table 2. Counts of *Enterobacteriae* in milk before and after ultrasound treatments

Samples	Enterobacteriae (log CFU mL^{-1})	Reduction (log CFU mL^{-1})
R	4.08	-
A1	1.86	3.22
A2	2.34	2.74
A3	2.68	2,40
A4	1.98	3.10
A5	1.60	3.48
A6	2.21	2.87
A7	1.01	4.07
A8	1.73	3.35
A9	1.8	3.28
A10	1.59	3.49
A11	1.63	3.45
A12	1.73	3.35
A13	2.98	2.10
A14	2.14	2,94
A15	1.68	3.40
A16	1.06	4.02

Table 3. D-values after ultrasound treatments at amplitudes 60, 90 and 120 μm for *Enterobacteriae* in milk

Microbes	Temperature	Decimal reduction time (min)		
	(°C)	D_{60}	D_{90}	D_{120}
Enterobacteriae	20	13.35	12.97	12.78
	40	10.13	10.01	9.89
	60	3.40	3,12	2.85

(20 °C) during 6 minutes is not efficient criteria for count of *Enterobacteriae* determined by Regulation for quality of milk and milk products (NN, 2001). However in sample A4 (treated at 120μm: 6 min: 60°C) plate count of *Enterobacteriae* was 1.98 log cfu mL^{-1} what is bellow of maximal acceptable limit determined by Regulation (Table 2). Also it was established that after longer ultrasound treatment (12 minutes) at ambient temperature (20 °C) reduction of *Enterobacteriae* was significant. Sample A11 was treated by ultrasound amplitude 120 μm and plate count was 1.63 log CFU mL^{-1} and sample A1 treated by amplitude 60 μm contain count of *Enterobacteriae* 1.86 log CFU mL^{-1} (Table 2). Higher temperature of milk treatment increased reduction of *Enterobacteriae*. Maximal inactivation of *Enterobacteriae* in milk was after ultrasound treatment by amplitude 120 μm during 12 minutes at 60 °C (Sample A7) (Table 2).Some authors have suggested that the efficacy of ultrasonic treatment for killing of bacteria by cavitational effects could be minimised with an increase in temperature. This fact could probably be the result of an increased thermal effect that would masque the effect of sonication, and/or a decrease of the violence of implosion due to the increased vapour pressure at higher temperatures. More bubbles are formed but these are smaller and the violence of implosion decreases (Allinger, 1975; Guerrero et al., 2001; Sala et al., 1995). This behaviour is not in agreement with results in this research. Although the cavitation effect could be minimised by the increase of temperature, in the case of milk, the concentration of solids in suspension could play an important role and improve the cavitation intensity (Sala at al., 1995; Villamiel and de Jong, 2000). Garcia et al. (1989) observed that at high temperatures the advantages of thermoultrasonication for killing of bacteria were maintained in milk since the z-values of thermoultrasonication and thermal destruction were very similar. Although the mechanism is not clear, they attributed these results to the concentration of solids presented in milk. Ciccolini et al. (1997) studied the survival of *S. cerevisiae* suspended in water at 45, 50 and 55 °C at different ultrasonic powers, and found that application of ultrasonic waves at non-lethal temperature (45 °C) did not display a deactivation action while synergy between ultrasound and heat was confirmed at the higher temperatures.

Efficacy of cavitations phenomena and micro streaming to inactivation *Enterobacteriae* in milk is possible monitor as ultrasound intensity by Margulis and Maltsev equation (2003) where intensity of applied ultrasound is represented as power of probe per square unit (Wcm^{-2}) respectively as emitted energy in J (Table 1). In table 3 it is obviously that time for decimal reduction (D_{us}) and also log of reduction (Table 2) for specific amplitude (60, 90 and $120_{\mu m}$) of ultrasound is in proportion with accepted energy respectively applied intensity (Table 1). At the lowest temperature (20 °C), the D values were between 13.35 and 12.78 for *Enterobacteriae* depending on the applied wave amplitude (60, 90 or $120_{\mu m}$). D-values for the thermosonification treatment decreased by approximately up to 20 % for *Enterobacteriae* when the temperature of treatment was increased to 40 °C (depending on the wave amplitude), as compared with corresponding values at 20 °C. Decimal reduction time was smallest at ultrasound treatment at amplitude of 120 μm (D_{120}) and temperature of 60 °C (Table 3). At those points, intensity of applied ultrasound was maximal 54.59 W/cm^2 but delivered energy was 60.125 J (Table 1). Scarce information is found in the literature on the influence of wave amplitude on microorganism inactivation. It has been reported that the intensity of ultrasound effect is directly related to the amplitude: when ultrasound amplitude increases, the zone undergoing cavitation increases, leading to more inactivation (Guerrero et al., 2001; Noci et al., 2009; Patist and Bates, 2008, Patil et al., 2009).

Table 4. Analysis of Variance for *Enterobacteriae* count after ultrasound treatment

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-value
A:amplitude	0.08649	1	0.08649	7.52	0.0336
B:treatment time	2.54016	1	2.54016	220.92	0.0000
C:temperature	1.17649	1	1.17649	102.32	0.0001
AA	0.0122277	1	0.0122277	1.06	0.3422
AB	0.0078125	1	0.0078125	0.68	0.4413
AC	0.0078125	1	0.0078125	0.68	0.4413
BB	0.0648981	1	0.0648981	5.64	0.0551
BC	0.0003125	1	0.0003125	0.03	0.8745
CC	0.0101004	1	0.0101004	0.88	0.3848
Total error	0.068988	6	0.011498		
Total (corr.)	3.99398	15			

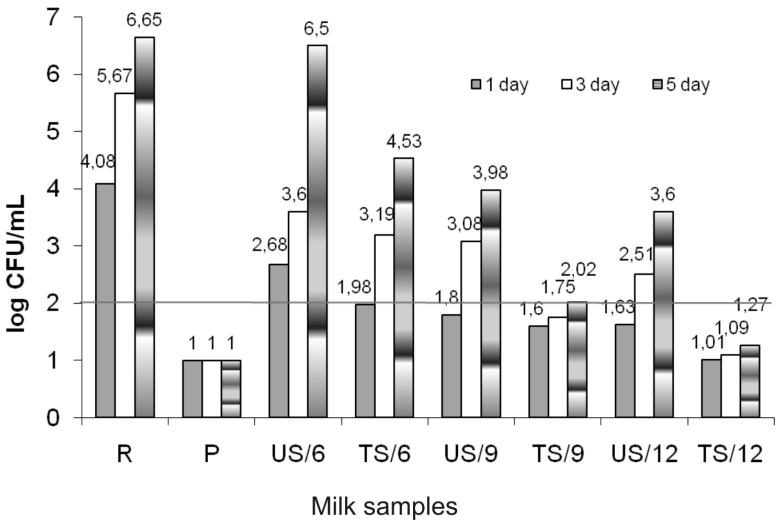
R-squared = 98.2727 percent; R-squared (adjusted for d.f.) = 95.6817 percent; Standard Error of Est. = 0.107229; Mean absolute error = 0.0540086; Durbin-Watson statistic = 2.16345 (P=0.7037)

Table 5. Optimised values of specific ultrasound parameters defined by Statgraphics where lowest *Enterobacteria* count was found

Factor	Low value	High value	Optimum value	Lowest value of *Enterobacteria* count (log CFU mL^{-1})
Treatment time (min)	6.0	12.0	12.0	1.06151
Amplitude (μm)	60.0	120.0	120.00086	
Temperature (°C)	20.0	60.0	60.0	

The shelf life of milk processed by ultrasound or termoultrasound treatment was evaluated and the results are shown in Fig. 1. Pasteurized milk (P) treated in plate heat exchanger at temperature 85 °C (15 sec) had *Enterobacteriae* count in frame of sanitary standards (NN, 2001) during the storage period of 5 days. During the storage period, the increase in *Enterobacteriae* count was higher for milk samples treated with ultrasound at ambient temperature (20 °C) (samples marked as US) as compared with corresponding values at higher temperature 60 °C (samples marked as TS), as shown in Fig. 1. From the data in Figure 1 it is clear that milk treated at 20 °C (Samples US/6, US/9 and US/12), already after 3 days of storage at temperature 4 °C contained higher count of *Enterobacteriae*. The count excides maximally accepted level by national sanitary standards for milk (2.0 log CFU mL^{-1}) (NN, 2001). Sample TS/6 treated at 60 °C meet criteria of national sanitary standards for milk but after 3 days of storage count of *Enterobacteriae* increased to 3.19 log CFU mL^{-1}. Milk treated by combination of temperature and ultrasound during 9 minutes (TS/9) after 5 days was not acceptable for consumption. However, ultrasound application for 12 minutes at 60 °C makes milk in frame of sanitary standards after 5 days shelf life.

The facts that substantially affect the inactivation of *Enterobacteriae* using ultrasound are the amplitude of the ultrasonic waves, the exposure/contact time with the microorganisms, and the temperature of treatment. The estimated effects of each variables and analysis of variance for the model are presented as Pareto chart in Figures 1 and 2. According to the ANOVA table, the fitted model was significant at consider confidence level since the F-value was more than three times that of the listed F-value (Table 4). In order to determine if an effect is significant or not, values of the column P-value in table 4 can be observed. Indeed, a P-value lower than 0.05 indicate that the considered factor is significant for the count of *Enterobacteriae* in milk. As one can see from the Pareto chart (Fig. 2), lines that are going over the vertical blue line are statistically



R - raw milk; **P** - pasteurized milk; **US/6** - sonicated milk - 6 minute; **TS/6** - termosonicated milk (60 °C) - 6 minute; **US/9** - sonicated milk - 9 minute; **TS/9** - termosonicated milk (60 °C) - 9 minute; **US/12** - sonicated milk - 12 minute; **TS/12** - termosonicated milk (60 °C) - 12 minute

Figure 1. Influence of sonification, termosonification and pasterization treatment on *Enterobacteria* inactivation in milk

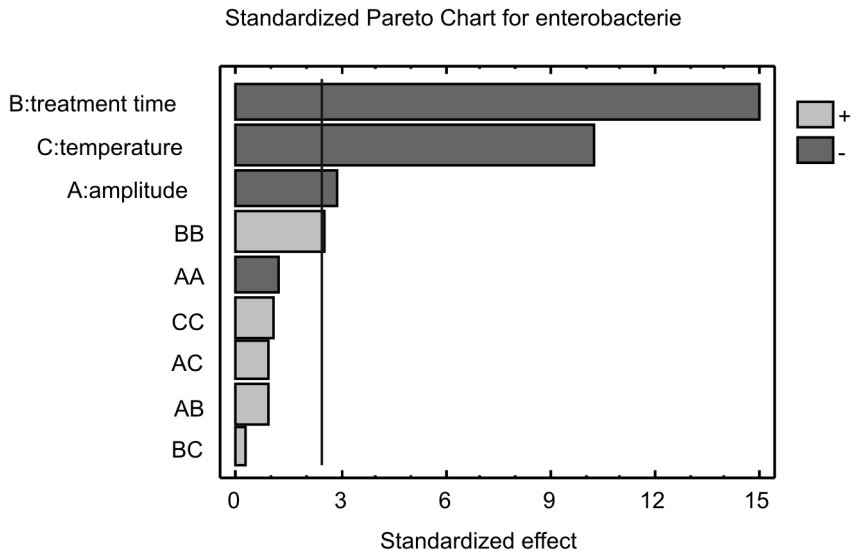
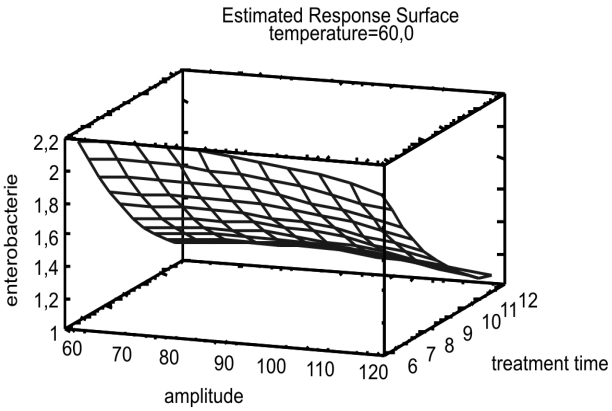


Figure 2. Standardized Pareto Chart for *Enterobacteriae* count after ultrasound treatment

significant factors influencing for the count of *Enterobacteriae* in milk (Fig. 2).

Surface plot for the count of *Enterobacteriae* are given in Figure 3. At fixed (optimised) values of temperature, surface plot is function of treatment time and amplitude. From the plot one can observe that the count of *Enterobacteriae* is lowest as optimised for the treatment time of 12 minutes, temperature (60 °C) and amplitude (120 μm) (Table 5). For shorter treatment time and lowest temperature count of *Enterobacteriae* is highest. This is very logical because for shorter treatment there are not enough cavitation phenomena and micro streaming that can break cell walls of bacteria and in that way

Figure 3. Surface plot for total *Enterobacteriae* count at optimise values at temperature (60 °C)



kill bacteria. Output optimal value of count of *Enterobacteriae* (1.06151 log CFU mL^{-1}) has been defined by Statgraphics where count for bacteria was as follows for specific ultrasound parameters: temperature, amplitude and treatment time.

Conclusion

The aim of the simultaneous use of the combined effect of ultrasound and temperature on *Enterobacteriae* inactivation was to reduce the temperature and/or the process time of sterilisation processes. Results of this investigation of combination effects of ultrasound and heat treatment compared to temperature or ultrasound treatment alone shows improved inactivation of *Enterobacteriae* in milk when using ultrasound plus heat treatment. The fact that seem to be crucial for inactivation of *Enterobacteriae* in milk using ultrasound are the amplitude of the ultrasonic waves, the exposure/ contact time with the microorganisms, and the temperature of treatment. The achieved results indicate significant inactivation of microorganisms under longer period of treatments with ultrasonic probe particularly in combination with higher temperature and amplitude. Output optimal value of total bacteria count has been defined by Statgraphics where lowest *Enterobacteriae* count (1.06151 log cfu mL^{-1}) was as follows for specific ultrasound parameters: amplitude of 120 μm, treatment time for 12.0 min and temperature of 60 °C.

Inaktivacija Enterobakterija u mlijeku ultrazvukom visokog intenziteta

Sažetak

Ultrazvuk kao netermalna metoda procesiranja hrane ima svoje prednosti pri inaktivaciji mikroorganizama u hrani u odnosu na klasične metode procesiranja hrane budući da uzrokuje manje negativnih učinaka npr. gubitak važnih nutrijenata te aroma. U ovom radu ispitivana je inaktivacija Enterobakterija u mlijeku primjenom ultrazvuka visokog intenziteta. Sirovo mlijeko sadržaja masti 4 %, tretirano je ultrazvučnom sondom promjera 12 mm i frekvencije 20 kHz. Eksperiment je planiran i proveden koristeći kompjuterski program STATGRAFICS (Centre composite design - CCD). Ultrazvučna obrada provedena je prateći tri parametra - temperaturu (20, 40 i 60 °C), amplitudu (120, 90 i 60 μm) te vrijeme procesiranja (6, 9 i 12 minuta). Sve analize provedene su neposredno nakon sonificiranja te nakon 3 odnosno 5 dana skladištenja uzoraka u hladnjaku na temperaturi od 4 °C. Inaktivirajući učinak ultrazvuka na mikroorganizme (Enterobakterije) ovisio je o primijenjenoj amplitudi ultrazvuka, vremenu i temperaturi procesiranja mlijeka. Dobiveni rezultati pokazuju značajan inaktivirajući učinak ultrazvuka kod dužeg perioda obrade ultrazvučnom sondom u kombinaciji sa povišenom temperaturom i amplitudom od 120 μm. Optimalna vrijednost (najmanja) Enterobakterija (1,06151 log cfu mL^{-1}) u mlijeku određena je Statgraphics-om pri sljedećim uvjetima obrade ultrazvukom: amplituda 120 μm, vrijeme obrade 12 minuta i temperatura od 60 °C.

Ključne riječi: ultrazvuk visokog intenziteta, mlijeko, *Enterobakterije*, D-vrijednost

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