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Influence and comparison of thermal, ultrasonic and thermo-sonic treatments on microbiological quality and sensory properties of rennet cheese whey

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Summary

Ultrasonication and thermo-sonication belong to alternative, non-thermal food processing methods. The aim of this study was to investigate the influence of different ultrasound power inputs (240 W, 320 W, 400 W) without and in combination with heat pre-treatment on microbial inactivation and sensory properties of rennet cheese whey in comparison with conventional pasteurization batch processes. Ultrasonication treatments had no impact on reduction of any group of studied microorganisms. Microbial inactivation caused by thermo-sonication treatments with pre-heating to 35 °C or 45 °C increased with nominal power input and/or exposure times and was probably due to the heat improved ultrasonic cavitation. Thermo-sonication treatments at nominal power input (400 W) and preheating to 55 °C were the most effective resulting in greater microbial reduction compared to that observed by simulating pasteurization processes, but occurred probably due to developed heat solely. Sensory properties after ultrasonication and thermo-sonication were considerably improved in comparison with that after simulated pasteurization processes. Mouth feel of whey samples was considerably better, there was no occurrence of sediment and colour remained unchanged in almost all samples.

Key words: ultrasonication, thermo-sonication, sensory properties, microbial inactivation, rennet cheese whey

Introduction

Whey is a by-product of cheese production. Liquid whey consists of approximately 93 % water, 4.5 % lactose and 1 % proteins. In smaller amounts minerals, vitamins and milk fat are also present (Jeličić et al., 2008).

Due to a high content of essential amino acids, whey proteins have a much higher biological and nutritional value than other proteins of animal origin. In recent years it has been proved that whey proteins also show some bioactive effects like antimicrobial, anti-hypertensive and anticarcenogenic properties (Režek-Jambrak et al., 2008). Besides, whey proteins have excellent functional properties i.e. foaming ability, solubility, emulsifying properties (Brnčić et al., 2008; Jeličić et al. 2008).

However, whey proteins tend to precipitate at temperatures above 60 °C (Parris et al., 1991). The high water content and high content of sugar in the dry matter make fresh whey very susceptible to microbial spoilage so heat treatments, i.e. pasteurization, are obligatory. As a result of heat treatment, sediments of proteins and some salts as well as redundant sourness are likely to occur (Jeličić et al., 2008). In such a manner nutritional and sensory

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quality of whey is reduced. Therefore whey is rarely processed into food products like beverages. It is mostly used as animal feed or processed into whey powder, whey protein concentrates and isolates by applying processing techniques with high energy consumption (Brnčić et al., 2011).

In recent years, a lot of effort has been put into investigation and development of new methods for food processing that have reduced impact on the nutritional content and quality of food, but significantly reduce microbial activity. The amount of required heat could thereby be partially reduced or considerably eliminated (Ugarte-Romero et al., 2007).

Ultrasonication belongs to this group of methods and can be defined as sound waves with a frequency higher than 20 kHz. Ultrasound has a number of applications in the food industry which can be divided into applications of low and of high energy ultrasound. Low energy ultrasound has intensities below 1 Wcm^{-2} and frequencies above 2-3 MHz. It is used for non-invasive purposes like foodstuffs characterization, determination of unwanted foreign bodies in raw materials and final products, level measurements in tanks etc. (Režek Jambrak et al., 2010). High energy ultrasound with intensities above 1 Wcm^{-2} and frequencies between 18 and 100 kHz found its possible application also in the dairy industry for processes like milk homogenisation, cleaning, enzyme and bacteria inactivation, chymosin and β-galactosidase extraction (Režek-Jambrak et al., 2009; Jeličić et al., 2010; Bosiljkov et al., 2011). The main active force during ultrasonication is of mechanical nature and causes the formation and implosion of bubbles in a liquid, what is known as cavitaton (Brnčić et al., 2010). During implosions, very high temperatures and pressures occur, reaching up to 5500 °C and 50 MPa, which is believed to be the main bactericidal effect of ultrasound (Villammiel and de Jong, 2000; Piyasena et al., 2003). That effect is mostly localized; it does not affect a large area and has not enough potential to destroy most resistant spores and bacteria. Consequently ultrasound treatments were not accepted as a food preservation method (Raso et al., 1998; Piyasena et al., 2003). More recent studies report a better lethal effect of ultrasound when combined with heat (thermosonication or thermoultrasonication). Many authors have observed good results in reduction of potential pathogens number like *Escherichia coli* (Lee et al., 2009), *Listeria monocytogenes* (Pagan et al., 1999; Ugarte-Romero et al., 2007) and *Shigella boydii* (Ugarte-Romero et al., 2007) as well as some non-pathogens like *Listeria innocua* (Noci et al., 2009; Bermudez-Aguirre et al., 2009). However, those studies were conducted either on a model solutions or raw milk where the concentration of the target microbe was known. However, there are no available studies about the impact of thermo-sonication on the overall microbiological quality of fresh whey.

The aim of this study was to investigate whether thermo-sonificaton could be used as an alternative processing method for the purposes of adequate microbiological quality assurance and improving sensory properties of rennet cheese whey. Therefore the influence of different ultrasound power inputs without and in combination with heat pre-treatment on microbiological quality of whey in comparison with conventional pasteurization processes were evaluated. Furthermore, sensory properties i.e. odour, flavour, appearance and colour were compared after ultrasonication, thermo-sonication and conventional pasteurization processes.

Materials and methods

Whey samples

Samples of fresh unpasteurized sweet whey were purchased from Vindija d.d. Dairy Industry (Varaždin, Croatia) and were stored at temperatures below 10 °C. The total solids amount was determined by the standard method of drying to constant mass at 102±2 °C (Carić et al., 2000). The amount of lactose was determined by a referent method using Na-tungstate and chloramine T in accordance with the International Dairy Federation (IDF) (Carić et al., 2000). The protein amount was determined by the Schulz method (Božanić et al., 2010), the mineral content by a method according to IDF (Carić et al., 2000) and the milk fat content by Gerber's butirometric method (Božanić et al., 2010). Titrable acidity was determined by the Soxhlet Henkel method (Božanić et al., 2010).

Storage and thermal treatment of whey samples

After receiving the whey samples from the supplier, 2 L of untreated rennet cheese whey was immediately poured into two sterile glass bottles, closed and placed in the refrigerator. Before using it

for microbiological and sensory analysis, 100 mL of whey was poured into a sterile glass vial, stoppered, placed into a thermal controlled water bath with constant shaking and gradually heated to a room temperature (approximately 20 °C). The temperature of the heating bath was maintained at 20±1 °C to allow heat transfer. The temperature of whey was monitored with a thermometer placed into a glass vial.

In addition, for comparison with thermo-sonication and ultrasonication, equal volumes of 100 mL of fresh whey were thermally treated at 72±1 °C for 20 seconds and at 65±1 °C for 30 min. These particular regimes were chosen to simulate the conventional batch pasteurization processes. Heating was performed in sterile closed glass vials placed into a thermal controlled water bath with constant shaking to assure homogenous dispersion of heat inside the samples throughout treatment. The temperature of the heating bath was maintained at 72±1 °C or at 65±1 °C to allow heat transfer. The temperature of whey was monitored with a thermometer placed into a glass vial. After finishing pasteurization, samples were gradually cooled down to approximately 20 °C.

Aliquots of each control sample were transferred to test tubes with physiological solution for further microbiological analysis. The rest of each sample was prepared for sensory evaluation as listed below. The experiment was conducted in triplicate.

Ultrasonic and thermo-sonic treatments of whey

Fresh whey samples (100 mL) were placed into sterile glasses with a total volume of 300 mL which were used as treatment vessels. An ultrasonic device (Hielscher Ultrasonics GmbH, Germany, model UP400S, amplitude regulation 20-100 %, frequency 24 kHz) with a 7 mm diameter probe was used for this research. The ultrasonic probe was directly immersed in the sample with depth of 1.0 cm from the surface. Ultrasonication was performed at 100 %, 80 % and 60 % of nominal output power, where the power level of 100 % corresponded to approximately 400 W, and the power levels 80 % and 60 % corresponded to approximately 320 and 240 W. Exposure times were 5, 6.5 and 8 min whereby the samples were tempered at room temperature (20 °C) before each treatment. Thus, there were a total of 9 ultrasonication treatments, which were denoted as 100 %/5' (5 min at power level 100 %), 100 %/6.5' (6.5 min at power level 100 %) etc. Thermosonication treatments were performed under the same conditions as mentioned above, only with the difference of preheating the samples to three different temperatures (35 °C, 45 °C, 55 °C). Thus, there was a total of 27 treatments which were denoted as 100 %-35 °C/5' (5 min. exposure at power level 100 % with preheating to 35 °C), 100 %-35 °C/6.5' (6.5 min exposure at power level 100 % with preheating to 35 °C) etc. Throughout each treatment, the temperature of the samples was monitored by an IR-thermometer (Raytek - MiniTemp FS, Raytek, Melrose, USA) and recorded in periods of 20 seconds. After each treatment aliquots were transferred to tubes with (containing) physiological solution for microbiological analysis. The rest of each sample was prepared for sensory evaluation as listed hereafter. Each experiment was performed in triplicate.

Acoustic energy density and ultrasound intensity determination

Acoustic energy density (AED) and ultrasound intensity (UI) values were determined using a calorimetric method (Tiwari et al., 2008a; 2008b). Whey samples were sonicated with temperature (T) recorded as a function of time under adiabatic conditions using an IR thermometer (Raytek - MiniTemp FS, Raytek, Melrose, USA). The initial temperature rise (dT/dt) was determined by polynomial curve fitting. Since there were no available data on the specific heat of whey, it was calculated using results observed by chemical composition analysis of whey, as shown in Eq (1):

$$C_{p=} w_v C_{pv} + w_c C_{pc} + w_p C_{pp} + w_f C_{pf} + w_a C_{pa} \quad (1)$$

$w_{v,}\ w_{c,}\ w_{p,}\ w_{f,}\ w_a$ = mass contents of the main whey constituents

$C_{pv,}\ C_{pc,}\ C_{pf,,}\ C_{pp,}\ C_{pa}$ = specific heat of the main whey constituents (Lelas, 2006).

Ultrasonic power (P), AED (WmL^{-1}) and UI (Wcm^{-2}) values were calculated using Eqs. (2), (3) and (4):

$$P = m * C_p * (dT/dt)_{t=0} \quad (2)$$

$$AED = P/V \quad (3)$$

$$UI = 4P/\pi D^2 \quad (4)$$

(dT/dt) = change in temperature over time (°C s^{-1}),

C_p = specific heat of whey (3.9365 kJ kg^{-1} C^{-1}),

P =ultrasonic power (W),

m = sample mass (kg),

V = sample volume (mL)

D = probe diameter (0.7 cm).

Microbiological analysis

All microbiological analysis was performed in accordance with Koch's pour plate method (Schlegel, 1992). Thereby, after each treatment serial dilutions of whey solution (0.9 % NaCl) were formed using physiological solution in order to evaluate the total viable cells count, the viable count of coliforms and the viable cell count of yeasts and moulds. As control samples, serial dilutions of untreated fresh whey, pasteurized whey and ultrasonicated whey were created. Furthermore, samples for the evaluation of the total viable cells count were pour-plated in Tryptic Glucose Agar (Biolife, Italy) and incubated at 30 ºC for 72 h. Coliform bacteria samples were pour-plated in Endo Agar (Biolife, Italy), while dishes were incubated at 37 °C for 48 h and bacteria loads were counted. For the enumeration of the viable yeasts and moulds count samples were pour-plated in Sabouraud-Maltose Agar (Biolife, Italy) and incubated at room temperature for 72 h. All microbiological analyses were conducted in a triplicate for each experiment.

Sensory analysis

Samples of untreated, thermally treated, sonicated and thermo-sonicated whey were collected in sterile glass bowls, closed and stored in a cool environment for 24 hours. Before sensory analysis they were adjusted to a room temperature, encoded and equal quantities of each sample were poured into transparent cups. In a room designed according to ISO8589:2007 samples were then presented simultaneously to each of the five assessors trained to perform sensory evaluation of whey. Each sample was evaluated for colour, appearance, flavour and odour where each attribute could have been rated with notes from 1 to 5. The average note of each attribute was multiplied with a predetermined weighting factor which was observed by the Delphi method. In this manner, scores for each evaluated attribute were observed. By summarizing scores of each attribute a final score for each particular sample was obtained. The maximum score that one sample could obtain was 20 (Molnar and Örsi, 1982; Filajdić et al., 1988).

Results

Whey composition

The average composition of whey is given in Table 1. According to data available from literature (Jelen, 2003; Jeličić et al., 2008), all determined parameters are in range characteristic for rennet cheese whey.

Microbial inactivation

Changes in total viable cells count

Figures 1a-c demonstrate the reduction of the total viable cell count obtained by ultrasonication and thermo-sonication treatments in comparison with the reduction observed by simulation of conventional pasteurization processes.

In general, ultrasonication treatments showed no impact on the reduction of the total viable cells count regardless the applied amplitude of ultrasonic wave and exposure time. Some treatments, especially those of applying power level of 80 % (Figure 1b)

Table 1. Average composition of fresh rennet whey

n=5						
pH	°SH	Lactose (%)	Total protein amount (%)	Ash (%)	Total solids (%)	Milk fat (%)
6.16±0.22	5.92±0.30	4.89±0.06	1.71±0.20	0.57±0.01	5.14±0.05	0.17±0.05

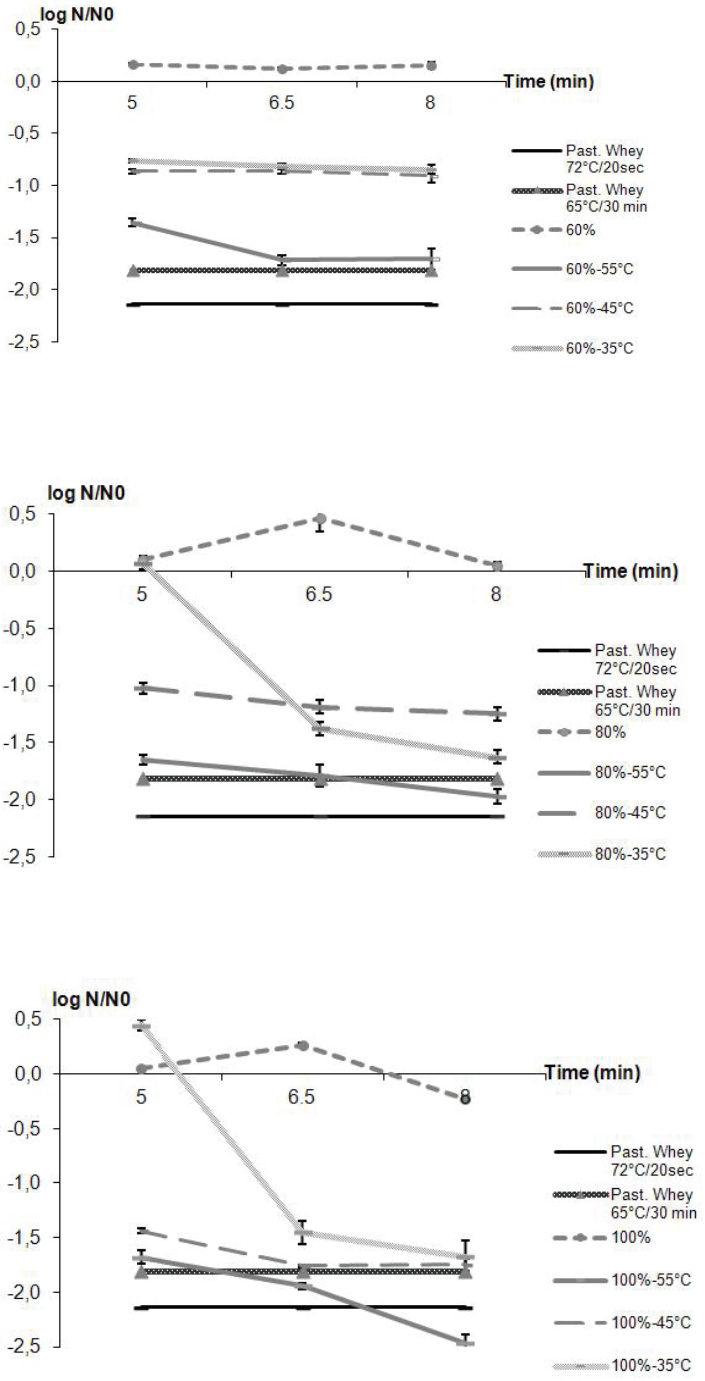

Figure 1. Reduction of total viable cells count by ultrasonication at a) 400 W (power level 100 %), b) 320 W (power level 80 %) and 240 W (power level 60 %) and by thermo-sonication with preheating to 55 °C, 45 °C and 35 °C during 5; 6.5 and 8 min of exposure in comparison to pasteurized whey

and of power level 60 % (Figure 1c), resulted even in a slight increase of the total viable cells count. This phenomenon might be the consequence of shattering the clusters of microbial cells without having a lethal effect at the same time. All applied thermo-sonication treatments resulted in the reduction of the total viable cells count where the observed reduction increased with exposure times and preheating temperatures.

As shown in Figure 1a, thermo-sonication treatments at power level 100 % (400 W) combined with preheating to 45 °C and 55 °C as well as exposure times of 6.5 min and 8 min resulted in a very good reduction of the total bacteria number. Thereby, thermo-sonication treatment denoted as 100 %-55 °C/8' resulted in a greater reduction of the total viable cells count (2.46 log cycles) in comparison with that observed by simulation of both pasteurization processes. Thermo-sonication treatment denoted as 100 %-55 °C/6.5' resulted in better reduction (1.94 log cycles) than simulated pasteurization process at 65° for 30 min (1.81 log cycles). The ultrasonic intensity of these treatments was calculated to be 64.42 W/cm^2 and 65.60 W/cm^2 which is much lower in comparison with intensities calculated for sole ultrasonication treatments for the same exposure times (Table 2b). However, data contained in Table 2a might provide an explanation for such results since during thermo-sonication treatments denoted as 100 %-55 °C/8' and 100 %-55 °C/6.5' temperatures of approximately 70 °C are developed. Previously it has been suggested that redundant increase of temperature could minimise the efficiency of killing bacteria by ultrasonic induced cavitation (Villamiel and de Jong, 2000). Raso et al. (1998) also found that at temperatures above 58 °C the inactivating effect is solely due to the heat. According to that and to the fact that whey samples were exposed

Table 2a. Change in temperature of whey samples during ultrasonication and termo-sonication treatments

Sample	Initial T (°C)	Final T (°C)	°T (°C)
Power level 100 % (400 W)			
100 %/5'	20.5±0.7	46.4±0.6	25.7±0.9
100 %/6.5'	20.6±0.7	50.6±0.7	30.0±0.9
100 %/8'	20.8±0.4	59.7±0.6	38.9±0.9
100 %-55 °C/5'	54.9±0.1	67.6±0.3	12.7±0.3
100 %-55 °C/6.5'	54.9±0.2	69±0.2	14.1±0.1
100 %-55 °C/8'	54.9±0.1	70.8±0.3	15.8±0.2
100 %-45 °C/5'	45±0.2	61.9±0.2	16.9±0.4
100 %-45 °C/6.5'	45±0.2	65.1±0.3	20.1±0.5
100 %-45 °C/8'	45±0.1	66.6±0.2	21.6±0.2
100 %-35 °C/5'	35±0.2	54.5±0.6	19.5±0.7
100 %-35 °C/6.5'	35±0.2	58.7±0.4	23.6±0.6
100 %-35 °C/8'	35±0.1	62.8±0.2	27.8±0.2
Power level 80 % (320 W)			
80 %/5'	20.2±0.8	38.5±0.6	18.1±1.0
80 %/6.5'	20.6±0.9	43.1±0.3	22.5±0.5
80 %/8'	20.8±0.6	52.6±0.5	31.8±0.9
80 %-55 °C/5'	55±0.1	62.5±0.8	7.4±0.8
80 %-55 °C/6.5'	55±0.1	63.8±0.7	8.7±0.7
80 %-55 °C/8'	55.1±0.2	66±0.3	10.9±0.2
80 %-45 °C/5'	45±0.1	57.9±0.3	13±0.4
80 %-45 °C/6.5'	45±0.1	60%.7±0.3	15.7±0.4
80 %-45 °C /8'	45±0.1	62±0.2	17±0.1
80 %-35°C/5'	35±0.2	50.2±0.3	15.2±0.4
80 %-35 °C/6.5'	35±0.2	53.1±0.3	18.2±0.3
80 %-35 °C/8'	35±0.1	55.4±0.4	20.5±0.4
Power level 60 % (240 W)			
60 %/5'	20.4±0.6	32.5±0.6	12.3±0.3
60 %/6.5'	20.5±0.5	36.5±0.9	16.0±1.4
60 %/8'	20.4±0.5	43.1±0.8	22.7±0.8
60 %-55 °C/5'	55±0.1	61.6±0.7	6.5±0.6
60 %-55 °C/6.5'	55±0.1	62.8±0.7	7.7±0.8
60 %-55 °C/8'	55.1±0.1	62.3±0.6	7.2±0.6
60 %-45 °C/5'	45±0.2	52±0.7	7.1±0.6
60 %-45 °C/6.5'	45±0.2	53.6±0.4	8.6±0.4
60 %-45 °C/8'	45±0.1	56.1±0.1	11±0.1
60 %-35 °C/5'	35±0.0	45.6±0.4	10.6±0.4
60 %-35 °C/6.5'	35±0.0	47.4±0.3	12.4±0.3
60 %-35 °C/8'	34.9±0.1	49.8±0.2	14.9±0.3

Table 2b. Calculated acoustic energy densities (AED) and ultrasound intensities (UI) for selected thermo-sonication treatments which resulted in greater microbial inactivation than simulated conventional pasteurization processes

Treatment	AED (W/mL)	UI (W/cm^2)	Microbial reduction better than pasteurization process at 72 °C/20 sec			Microbial reduction better than pasteurization process at 65 °C/30 min		
			Total viable cells	Coliform bacteria	Yeasts and moulds	Total viable cells	Coliform bacteria	Yeasts and moulds
Power level 100 % (400 W)								
100 %-55 °C/8'	0.25±0.03	65.60%±7.43	+	+	+	+	+	+
100 %-55 °C/6.5'	0.25±0.01	64.42±2.22	-	+	+	+	+	+
100 %-55 °C/5'	0.83±0.00	59.85±0.83	-	+	-	-	+	+
100 %-45 °C/8'	0.33±0.01	84.74±3.13	-	-	+	-	-	+
100 %-45 °C/6.5'	0.31±0.01	79.37±1.71	-	-	-	-	-	+
Power level 80 % (320 W)								
80 %-55 °C/8'	0.14±0.01	35.63±2.29	-	+	+	+	+	+
80 %-55 °C/6.5'	0.13±0.01	34.65±2.30	-	+	+	+	+	+
80 %-45 °C/8'	0.23±0.01	58.91±3.62	-	+	+	-	+	+
80 %-45 °C/6.5'	0.21±0.01	54.19±1.20	-	+	+	-	+	+
80 %-35 °C/8'	0.27±0.01	70.40±1.00	-	+	+	-	+	+
80 %-35 °C/6.5'	0.27±0.01	70.40±1.00	-	-	+	-	-	+
Power level 60 % (240 W)								
60 %-55 °C/8'	0.08±0.01	21.53±3.20	-	-	+	-	+	+
60 %-55 °C/6.5'	0.09±0.00	23.47±0.88	-	+	+	-	+	+
60%-55°C/5'	0.08±0.01	20.35±2.51	-	-	+	-	+	+
60 %-45 °C/8'	0.13±0.01	33.91±2.23	-	+	-	-	+	-
60 %-45 °C/6.5'	0.10±0.01	26.07±1.37	-	+	-	-	+	-

to temperatures above 70 °C for approximately one minute, the observed reduction of the total viable cells count at this specific treatments is due to heat rather than to ultrasound induced cavitation.

Thermo-sonication treatments at power level 80 % (320 W) combined with preheating to 55 °C and exposure times of 6.5 and 8 min resulted in the same or better reduction in comparison with simulated pasteurization batch process at 65 °C for 30 min (Figure 1b). According to data presented in Table 2a, the temperature of those samples increased up to approximately 66 °C. The calculated ultrasonic intensities remained half lower than in case of sole ultrasonication treatments at the same exposure times (Table 2b). Therefore the obtained results were probably due to the heat development during thermo-sonication treatment as Raso et al. (1998) previously suggested.

Moreover, it can be observed that reduction obtained by treatments with preheating to 35 °C and exposure times of 6.5 min and 8 min exceeded reduction obtained in treatments with preheating to 45 °C and same exposure times. Data contained in Table 2a address how temperature increase was somewhat higher during thermo-sonication treatments with preheating to 35 °C. That might have improved the bactericidal effect of ultrasonic induced cavitation. Additionally, this presumption is supported by comparison of the calculated ultrasonic intensities which are much lower in the case of thermo-sonication treatments combined with preheating to 45 °C as shown in Table 2b.

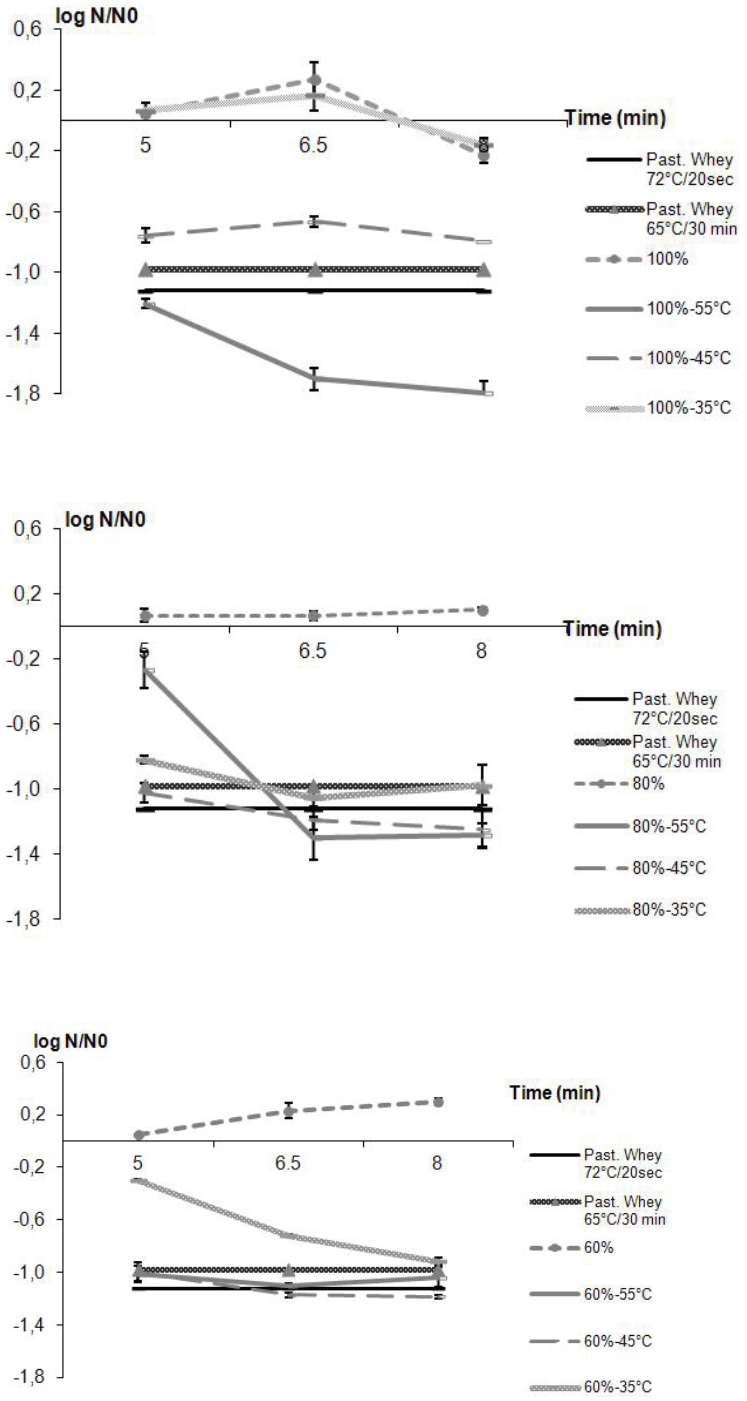

Figure 2. Reduction of viable coliform bacteria count by ultrasonication at a) 400 W (power level 100 %), b) 320 W (power level 80 %) and c) 240 W (power level 60 %) and by thermo-sonication with preheating to 55 °C, 45 °C and 35 °C during 5; 6.5 and 8 min of exposure in comparison to pasteurized whey

As shown in Figure 1c, neither of the thermo-sonication treatments at power level 60 % (240 W) resulted in better reduction of the total viable cells count in comparison with simulated pasteurization processes. However reduction obtained by treatments with preheating to 55 °C and exposure times of 6.5 min and 8 min reached very similar levels (approximately 1.7 log cycles) as simulated pasteurization batch process at 65 °C for 30 min (1.8 log cycles). It is notable that the temperature increase of these samples was not excessive (Table 2a), but the calculated ultrasonic intensities were more than half lower than in the case of ultrasonication treatments at the same exposure times. Consequently, heat pre-treatment probably caused an improvement of antimicrobial effect of ultrasonic induced cavitation.

Changes in viable coliform bacteria count

Figure 2a-c demonstrates reduction of viable coliform bacteria count obtained by ultrasonication and thermo-sonication treatments in comparison with reduction observed by simulation of conventional pasteurization processes.

Likewise in the case of total viable cell count reduction, ultrasonication treatments showed no impact on the reduction of the viable coliform cells count regardless the applied amplitude of ultrasonic wave and exposure time. Also, slight increase in viable cell count is evident, especially after ultrasonication at power level 60 %. According to data presented in Table 2a, the final temperature of the ultrasonicated samples ranged between

32.5±0.6 °C (exposure time 5 min) and 43.1±0.8 °C (exposure time 8 min). Coliform bacteria belong to mesophillic bacteria and prefer growth temperatures from 37 °C to even 49 °C (Fotadar et al., 2005). Thereat generation time can be as short as 20 minutes if the temperature is high enough (Smith, 1985). In accordance to that, utrasonication itself provides convenient growth temperatures for coliform bacteria, especially at power level 60 %.

As presented in Figure 2a, the best reduction was observed when applying power level 100 % and preheating to 55 °C. Thereby regardless the exposure times, the obtained reduction levels (1.20 to 1.79 log cycles) were higher than in the case of simulated pasteurization processes (0.98 to 1.12 log cycles). Heat development during these treatments resulted in the samples reaching a final temperature up to 71 °C, which implies that the inactivation effect was probably due to the heat solely as Villamiel and de Jong (2000) previously suggested.

Regarding thermo-sonication treatments at power level 80 %, reduction obtained after treatments with preheating to 45 °C and 55 °C and exposure times of 6.5 min and 8 min exceeded reduction obtained by simulation of both pasteurization processes, as shown in Figure 2b. Similar results were observed in treatments with preheating to 35 °C and exposure times of 6.5 min and 8 min as well as in treatment denoted as 80 %-45 °C/5' where the obtained reduction reached the same levels as simulated batch pasteurization process (65 °C for 30 min). According to data in Table 2a, heat development during these treatments might be responsible for such inactivating effects, which is in concordance with findings of Raso et al. (1998). However, unlike Raso et al. (1998), in the present study no additional hurdle such as pressure was applied. Additionally, from data presented in Table 2b, it is evident that the ultrasonic intensity of highlighted thermo-sonication treatments rises conversely to preheating temperature and final temperature of whey samples. In such a manner the obtained inactivating effect is most probably due to synergistic acting of heat and ultrasonic cavitation.

As presented in Figure 2c, all thermo-sonication treatments at power level 60 % in combination with preheating to 45 °C or 55 °C resulted in better reduction of viable coliform bacteria count in comparison with simulated batch pasteurization process (65 °C for 30 min). Thereat treatments denoted as 60 %-45 °C/6.5' and 60 %-45 °C/8' even exceeded reduction levels in comparison with simulated pasteurization process at 72 °C for 20 sec, although heat development did not result in final temperature increase above 56.2 °C (Table 2a). As previously discussed, the inactivating effect of thermo-sonication treatments at power level 60 % relays also most probably on heat improved ultrasonic cavitation.

Changes in viable yeasts and moulds count

Figure 3a-c demonstrates reduction in the viable yeasts and moulds count obtained by ultrasonication and thermo-sonication treatments in comparison with reduction observed by simulation of conventional pasteurization processes.

Neither ultrasonication treatment showed an impact on the reduction of the yeasts and moulds viable count regardless the applied amplitude of ultrasonic wave and exposure time.

As presented in Figure 3a, the best reduction was observed when applying power level 100 % and preheating to 45 °C or 55 °C. The obtained reduction during these treatments exceeded effects of simulated pasteurization batch process (1.34 log cycles) regardless exposure times. According to Table 2a, heat development during thermo-sonication at power level 100 % and preheating to 45 °C or 55 °C treatments resulted in final temperature of the samples ranging from 62 °C to 71 °C. This implies that the inactivation effect was probably due to heat solely as Villamiel and de Jong (2000) and Raso et al. (1998) previously suggested.

The increase of exposure time and preheating temperature has been accompanied with increase in the reduction level regarding thermo-sonication treatments at power level 80 %, as shown in Figure 3b. Furthermore, all thermo-sonication treatments with preheating to 55 °C as well as those with preheating to 45 °C or 35 °C and exposure times of 6.5 and 8 min resulted in better inactivation in comparison with effects obtained by simulation of both pasteurization processes. Relating to data in Tables 2a-b and to findings of Raso et al. (1998), as previously discussed in case of coliform bacteria reduction, the obtained effects relay on heat improved ultrasonic cavitation.

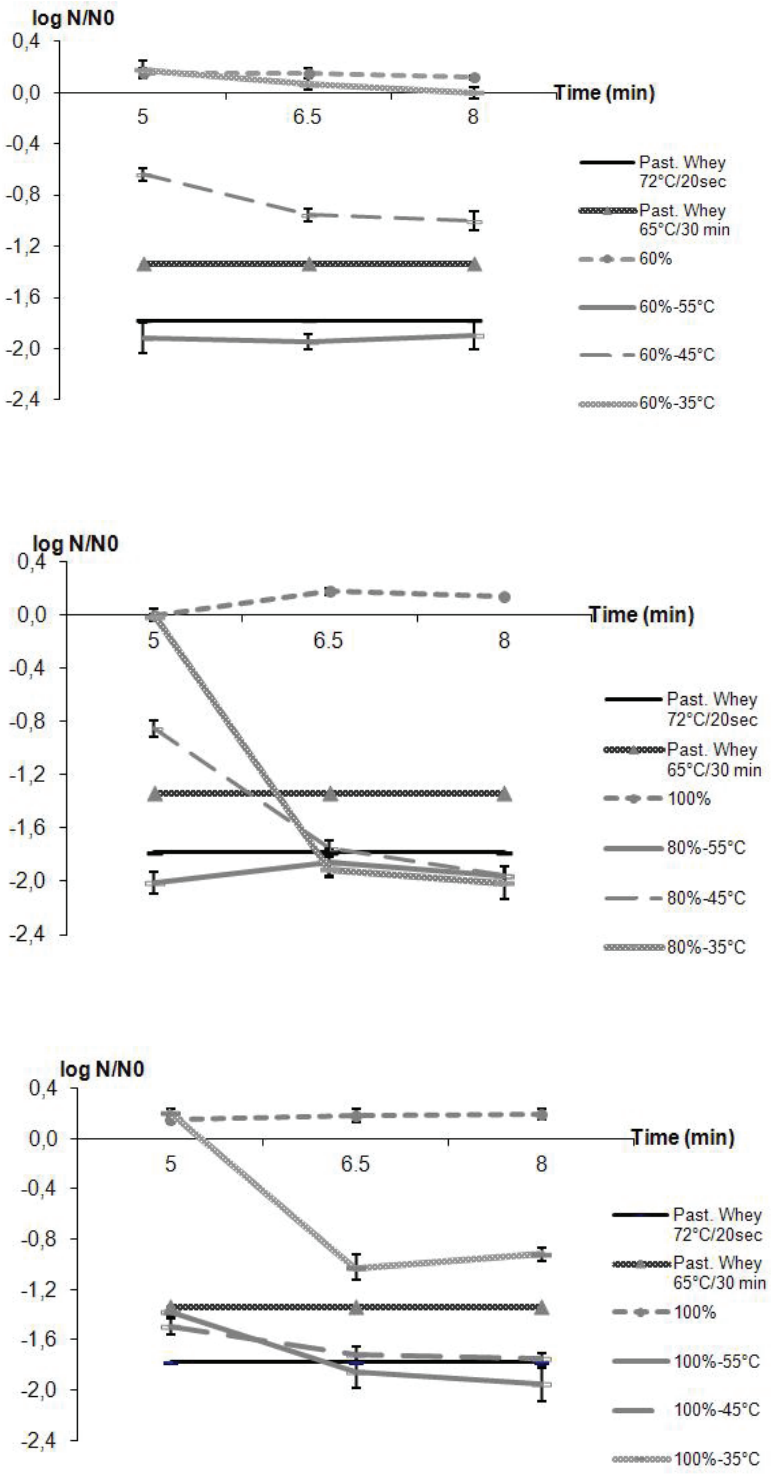

Figure 3. Reduction of viable yeasts and moulds count by ultrasonication at a) 400 W (power level 100 %), b) 320 W (power level 80 %) and c) 240 W (power level 60 %) and by thermo-sonication with preheating to 55 °C, 45 °C and 35 °C during 5; 6.5 and 8 minutes of exposure in comparison to pasteurized whey

According to Figure 3c, thermo-sonication at power level 60 % resulted in the best reduction when preheating to 55 °C was applied. Regardless exposure time, all mentioned treatments were more effective than simulated pasteurization processes.

Sensory evaluation

The results of the sensory evaluation of whey samples after thermo-sonication and ultrasonication in comparison with fresh and pasteurized whey are shown in Figures 4 a-c. In general, all sonicated and thermo-sonicated whey samples obtained higher average scores than fresh or pasteurized samples. Mouth feel of ultrasonicated and thermo-sonicated whey samples ultrasonication thermo-sonication was considerably better. There was no occurrence of sediment in any of those samples and colour remained unchanged in almost all samples. Režek-Jambrak et al. (2008) studied the influence of high intensity ultrasound on functional properties of whey proteins. The obtained results showed that ultrasonication significantly improved the solubility of all tested solutions. Additionally, the homogenization effect of ultrasound is also well known (Režek-Jambrak et al., 2009). These two phenomena - increase in whey protein solubility and homogenization effect - might be responsible for the general improvement of fresh whey sensory properties after ultrasonication and thermo-sonication.

Flavour was slightly sweeter while odours of cooked milk were not noticed. However, samples where power level of 100 % (400 W) was applied had considerably

brighter colour and a metallic aftertaste. This was especially intensive after each thermo-sonication treatment with preheating to 55 °C. Furthermore, all samples that were treated with power level 100 % in combination with preheating to 35 °C had poorer mouth feel in comparison with samples which were preheated to 45 °C or to 55 °C. Therefore, samples where power level 100 % was applied after preheating to 45 °C received the highest scores in sensory evaluation as shown in Figure 4a.

The same observations were noticed in samples where power level 80 % was applied in combination with preheating to 35 °C or 55 °C. However, these phenomena were less expressed why these samples received higher scores of sensory evaluation than in case above. As shown in Figure 4b, samples which were preheated to 45 °C received also in this case the highest scores in sensory evaluation.

As shown in Figure 4c, samples where power level 60 % was applied solely or in combination with preheating to 55 °C, received somewhat lower scores of sensory evaluation in comparison with samples which were preheated to 35 °C or to 45 °C. Samples treated solely by ultrasound had poor mouth feel and slightly expressed odour of cooked milk, while samples which were preheated to 55 °C had a metallic odour and rubbery aroma which is in correspondence with results obtained by Riener et al. (2009). They studied the influence of high intensity ultrasound on sensory properties of raw milk by determining volatile compounds developed in milk during ultrasonication. Volatiles detected by GC spectrometry were predominantly hydrocarbons and are probably of pyrolytic origin, possibly generated by high localized temperatures associated with cavitation phenomena. Some of the detected volatiles are believed to cause the occurrence of the rubbery aroma in milk, which was less intense by reduction of the sonication power from 400 W to 100 W.

Relating to all above mentioned observations, regardless the ultrasound power input, preheating to 55 °C results in occurrence of a metallic aftertaste in thermo-sonicated samples. Furthermore, preheating to 35 °C, as well as applying power level 60 % (240 W), resulted in somewhat poorer mouth feel in comparison to other thermo-sonicated and ultrasonicated samples.

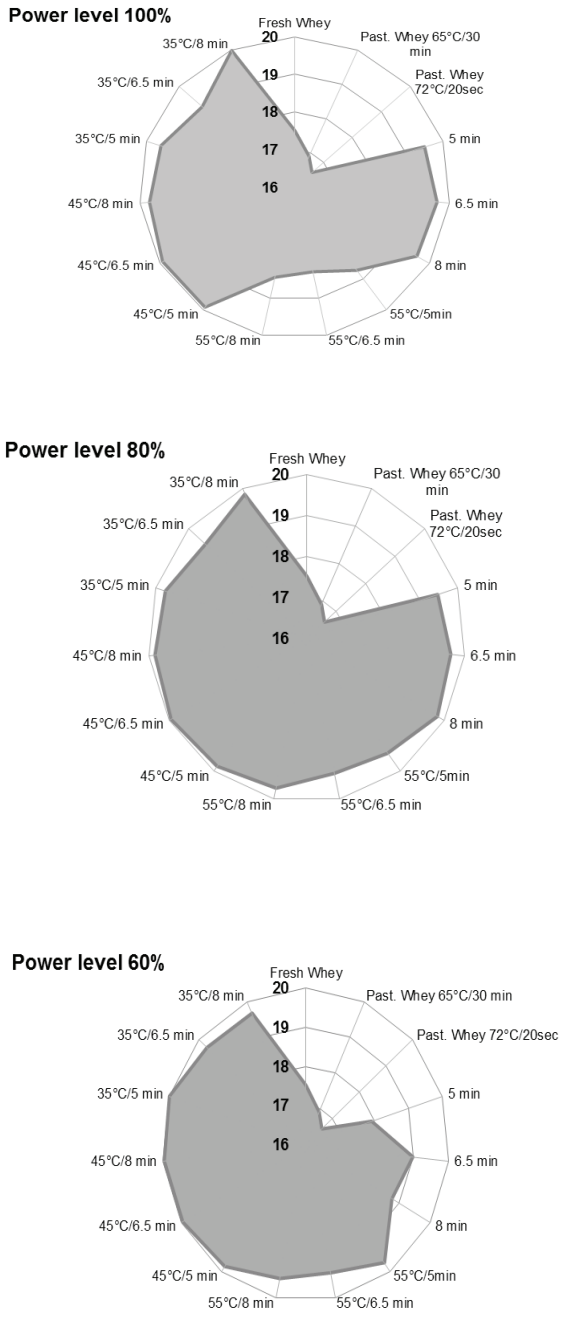

Figure 4. Sensory evaluation of whey samples after ultrasonication at a) 400 W (power level 100 %), b) 320 W (power level 80 %) and c) 240 W and after thermo-sonication with preheating to 55 °C to 55 °C, 45 °C and 35 °C during 5, 6.5 and 8 minutes of exposure compared to fresh and pasteurized whey

Conclusions

In general, the investigated ultrasonication treatments showed no impact on microbial inactivation regardless the ultrasound power input and exposure times.

Thermo-sonication treatments with pre-heating to 55 °C showed good impact on microbial inactivation but that effect is probably due to heat developed during the treatment.

Microbial inactivation caused by thermo-sonication treatments with pre-heating to 35 °C and 45 °C increased with power level and/or exposure times. Better effects than those obtained by simulation of conventional pasteurization processes are obtained at longer exposure times (6.5 and 8 min) and regarding reduction of coliform bacteria and yeasts and moulds.

Regarding calculated ultrasonic intensities and temperature increase of samples during these treatments, it can be concluded that the obtained microbial inactivation might be a result of heat supported ultrasonic cavitation. However, additional studies where same exposure times and power inputs would be applied at constant preheating temperatures should be carried out in order to support this thesis.

Ultrasonication and thermo-sonication treatments considerably improved sensory properties of rennet cheese whey in comparison with simulated pasteurization processes.

Thermo-sonication treatments at ultrasound power inputs of 400 W and 320 W in combination with preheating to 45 °C, regardless exposure time, appeared to be optimal for the purpose of the sensory properties of whey improvement.

Overall, it can be concluded that future studies should focus on processing parameters optimization at latter conditions in order to obtain adequate reduction of total bacteria count by applying longer exposure times and/or varying cycles.

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Utjecaj i usporedba toplinske obrade, ultrazvuka i termosonifikacije na mikrobiološku kvalitetu i senzorska svojstva slatke sirutke

Sažetak

Ultrazvuk i termosonifikacija ubrajaju se u alternativne, netermalne metode procesiranja hrane. Stoga je svrha ovog rada bila ispitati utjecaj različitih ulaznih snaga ultrazvuka (240 W, 320 W, 400 W) sa i bez primjene umjerenih temperatura na mikrobiološku kvalitetu i senzorska svojstva svježe slatke sirutke te usporediti s utjecajem toplinske obrade na iste parametre. Obrada ultrazvukom nije pokazala nikakav utjecaj na redukciju bilo koje skupine praćenih mikroorganizama. Tretmani termosonifikacije uz predgrijavanje uzoraka na 35 °C, odnosno 45 °C rezultirali su mikrobiološkom redukcijom koja je rasla proporcionalno trajanju tretmana i/ili ulaznoj snazi, a vjerojatno je posljedica kavitacije pojačane učinkom topline. Najuspješnijima su se pokazali tretmani termosonifikacije uz predgrijavanje uzoraka na 55 °C i primjenu ulazne snage 400 W te su rezultirali većom mikrobiološkom redukcijom nego simulirani postupci pasterizacije. Međutim, dobivena redukcija vjerojatno je posljedica djelovanja razvijene topline. Obrada ultrazvukom i termosonifikacijom rezultirala je značajnim poboljšanjem senzorskih svojstava sirutke u odnosu na simulirane postupke pasterizacije. Punoća okusa uzoraka sirutke bila je značajno bolja, nije uočena pojava taloga, a boja je ostala nepromijenjena u gotovo svim uzorcima.

***Ključne riječi*:** ultrazvuk, termosonifikacija, senzorska svojstva, mikrobiološka redukcija, slatka sirutka

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