Study on the development of lactic acid bacteria in cow’s and sheep’s milk treated with ultrasound

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ABSTRACT

Raw milk is a complex nutritional medium suitable for the growth of various microorganisms. Different methods such as pasteurization, bactofugation, etc. have been applied to eliminate undesirable bacteria. An alternative to these practices is the ultrasonic treatment of raw milk. An important advantage of this method is the destruction of bacterial cells without altering the vitamin content and fat, achieving at the same time good homogenization of milk. The acidification ability of Lactobacillus delbrueckii ssp bulgaricus and Streptococcus thermophilus cultured both in raw and pasteurized ultrasonic cow’s and sheep’s milk at 42°C and post-acidification of the cultures has been examined.

Introduction

The changes in consumers’ preferences as well as the need to produce safe and high-quality food underlie the improvement of the existing traditional production processes and the development of new ones. The introduction of new technologies aims at reducing the length of the production process and improving the conditions under which the individual operations are performed. The fulfillment of these requirements is related to the production of quality products and the preservation of the natural characteristics of the food. Another important issue is the reduction of energy consumption, which means reducing the damages caused to the environment and also decreasing financial costs (Carcel et al., 2011).

Over the last few years a number of surveys have been conducted concerning the use of ultrasound, high pressure, electric pulsations, magnetic field, etc. The ultrasound processing as well as its combination with mild pressure or temperature treatment has a number of advantages. It affects the efficiency of production and the quality of the end products which is expressed in reduction of the technological time, reduced chemical and physical risk, restriction of the negative effect on the environment, etc. (Chemat et al., 2011). The use of ultrasound, compared to some other traditional technological operations, allows producing high-quality food with maximally preserved original characteristics as well as having improved or even new functional properties (Ashokkumar et al., 2008).

Ultrasound is used in food industry in two main directions – for diagnostic purposes with a minimum effect on the tested product and for changing the main characteristics of the product. For diagnostic purposes high frequency ultrasound (several MHz) with low power (not higher than 1 W/cm2) can be used. Its use allows examining the raw materials during their processing and gives information on the quality of the obtained final products (Dukhin et al., 2005, Benedito et al., 2000, Elvira et al., 2005). The use of low-frequency (20-100kHz) and high-power ultrasound (10-1000 W/cm2) leads to physical, mechanical and chemical changes in the treated medium and can be used for optimizing the technological processes (Dolatowski et al., 2007, Carcel et al., 2011). This is one of the reasons for using ultrasound as an alternative in various technological processes for food production, for example for homogenization, grinding, mixing, pasteurization and separation of liquids and solid particles. Ultrasound treatment increases the effectiveness of the traditional processes like filtration, extraction, crystallization and fermentation (Patist and Darren, 2008, Leonelli and Mason, 2010).

In dairy industry ultrasound is used for homogenization of milk fat (Villamiel and Jong, 2000, Ertugay et al., 2004, Bermudes-Aguirre et al., 2008), examination of characteristics of the final products (Shopov et al., 2009), inactivation of enzymes (O’Donnell et al., 2010), bacteria (Cameron et al., 2009), etc. Heat treatment is still the most common method used in dairy industry due to its ability to destroy microorganisms and inactivate enzymes. This treatment
has been facilitated by the use of advanced apparatuses and their relatively easy maintenance. However, heat treatment has a significant effect on the source materials and causes a number of chemical and physical changes. These changes may deteriorate the organoleptic properties of the product and also impair the activity of certain substances (Soria and Villamiel, 2010). At the same time, the use of the so called “ultrasound pasteurization” at 50°C can preserve the quality of a number of foodstuffs with regard to their physical and chemical properties, their colour and smell compared to the traditional techniques for pasteurization which are conducted at higher temperature (Patist and Darren, 2008).

The use of ultrasound separately or in combination with a temperature as well as the respective parameters of the sound wave necessitates determining the changes happening in milk. The changes in the milk under the influence of ultrasound are irreversible (Zisu et al., 2010, Nguyen and Anema, 2010, M. Ashokkumar et al., 2009, Bermudes-Aguirre et al., 2009) and could influence the growth of lactic acid bacteria in processed milk.

The purpose of this research was to establish the influence of ultrasound processing of raw and pasteurized cow’s and sheep’s milk on the fermentation activity of the starter culture.

**Materials and methods**

**Experimental designs**

The parameters examined in this study included treatment time, milk type (raw and pasteurized sheep’s and cow’s milk) and lactic acid strains.

The following samples were prepared: raw sheep’s milk (RSM) and raw sheep’s milk processed by ultrasound for 1 minute (RSM1); pasteurized sheep’s milk (PSM) and pasteurized sheep’s milk processed by ultrasound for 1 minute (PSM1); The samples of the cow’s milk were the following: raw milk (RCM), raw cow’s milk processed by ultrasound for 1 (RCM1) and 3 (RCM3) minutes; pasteurized cow’s milk – control sample (PCM) and pasteurized cow’s milk processed by ultrasound for 1 (PCM1) and 3 (PCM3) minutes.

**Bacterial strains**

Two strains of *Lactobacillus delbrueksii ssp bulgaricus* and *Streptococcus thermophilus* were used in this experiment. The cultures were obtained from microbiological collections of Laktina Ltd., Sofia. The lyophilized cultures were activated by means of threefold re-planting onto sterile reconstituted dry milk media with 10% of dry substance and cultivated at 43°C. Working starter cultures were prepared from this sub-culture and serially diluted to yield the required concentration of 5x10⁶ CFU/ml.

**Milk parameters**

The experiments have been carried out with natural, non-homogenized cow’s and sheep’s milk. The cow’s milk was produced in private farms in the region of Plovdiv and the sheep’s milk was delivered from the farm of the Agricultural University. The main physical and chemical characteristics of the raw sheep’s milk are presented in table 1. The total titratable acidity is expressed in Torner degrees (°T) and means the quantity (ml) of 0.1N NaOH used for neutralizing 10 ml of milk which had been preliminarily diluted with distilled water in a ratio of 1:2 with phenolphthalein as an indicator.

The pasteurization of the milk was performed under 95°C for 30 minutes in a water bath, (Memmert, Germany). The milk had been initially pasteurized and then subjected to ultrasound processing.

**Power ultrasound treatment**

Samples (400ml) were sonificated in sterilized polypropylene bags using Ultrasonic Cleaner Sinus 2501, at a constant frequency of 42 kHz and working volume of 700 ml. The pulse durations of 1 and 3 minutes were applied.

**Statistical analysis**

The statistical processing has been conducted using the program SPSS version 9.0, with an analysis of the samples (ANOVA) based on the ultrasound processing factor and the criterion LSD, with a level of significance of 0.05.

**Results**

It is well known that lactic acid bacteria absorb the lactic sugar and produce lactic acid. During the cultivation of the microorganisms in the milk, the fermentation process was monitored by observing the changes of pH and the titratable acidity (Soukoulis et al., 2007). The data were obtained after conducting three independent experiments with two parallel samples. The dependences of titratable acid vs. time have been graphically presented using SigmaPlot 2001 for Windows 7.0.

**Effect of ultrasonic power on fermentation activity of sheep’s milk**

The obtained results for establishing the fermentation activity of the yoghurt starter culture in raw and processed by ultrasound sheep’s milk under 430°C show an increase in the titratable acidity are presented in Fig. 1.
After the first hour, the titratable acidity of the raw sheep’s milk and the milk processed by ultrasound is similar - 28°T ±1.22 and 28.7°T ±3.17. The average titratable acidity of the pasteurized sheep’s milk is higher, reaching 32°T ±3.42 in the control sample, while in the milk processed by ultrasound it is 30.5±3.2°T. The statistical processing of the results shows that after the one-hour cultivation under 43°T there is no significant difference in the titratable acidity of the control samples and the corresponding test samples of raw and pasteurized sheep’s milk with or without ultrasound processing.

After the 2nd hour of incubation, the lowest and statistically significant titratable acidity was measured in the raw sheep’s milk - 44.2°T ±0.92. As regards the other samples, higher titratable acidity was measured in those samples that were processed by ultrasound – raw milk - 56.75°T ±1.3 and pasteurized milk - 54.25°T ±2.43, while in the pasteurized sheep’s milk not processed by ultrasound the measured value was 50.75°T ±3.64. By the end of the incubation period, the samples of sheep’s milk processed by ultrasound have higher titratable acidity. The processed pasteurized milk has acidity of 112.5°T ±7.8 and the raw milk - 111°T ±8.1. The milk that was only pasteurized has acidity of 88.25°T ±1.31 and the unprocessed raw sheep’s milk - 87.5°T ±0.96. The statistic analysis shows that during the 3rd hour there is a significant difference between the established titratable acidity of the samples processed by ultrasound compared to those that were not processed regardless of whether the milk underwent heat treatment or not. This shows the better development of the lactic acid bacteria in sheep’s milk processed by ultrasound.

**of ultrasonic power on fermentation activity of cow’s milk**

The data on the changes of the titratable acidity during the incubation of the end product in processed and unprocessed cow’s milk shows that the ultrasound processing does not have an inhibitory effect on the microorganisms (Fig. 2).

After the one-hour incubation, the titratable acidity of the raw cow’s milk is the highest in the sample processed by ultrasound for 3 minutes – 30.5°T ±4.63, followed by the sample processed for 1 minute - 29.25°T ±3.9, while the measured value for the unprocessed raw milk was 28.25°T ±3.32. The titratable acidity of pasteurized milk is again higher in those samples that were processed by ultrasound: in the sample processed for 3 minutes - 33.5°T ±6.2 and in the sample processed for 1 minute - 33.25°T ±1.6, while the measured value in the control sample was 28.50°T ±2.72.

After the 2nd hour of incubation, the titratable acidity increased at the same rate in all the samples – by 15°T on average and no statistically significant difference could be observed. The samples that were processed for 1 minute have the highest titratable acidity: raw cow’s milk – 51.5°T ±8.14 and pasteurized cow’s milk – 49.25°T ±2.32. The unprocessed raw cow’s milk has the lowest titratable acidity - 38°T ±3.8. The titratable acidity is similar in the raw milk processed by ultrasound for 3 minutes - 44.2°T ±6.94, the unprocessed pasteurized milk – 43.25°T ±1.97 and also in the milk processed by ultrasound for 3 minutes – 46.5°T ±1.76.

By the third hour, the titratable acidity increased by 37°T on average in all samples, which indicates an intensive process of acid formation. The lowest titratable acidity after the third hour was measured in the unprocessed raw cow’s milk – 72.5°T ±2.63, which statistically distinguishes it from the sample processed by ultrasound for 3 minutes (80.75°T ±0.48) but not from the sample processed for 1 minute (77.50°T ±0.5). The samples of pasteurized cow's milk have higher average titratable acidity compared to the samples of raw milk. The pasteurized cow’s milk processed for 1 minute has titratable acidity of 90.25°T ±2.29 after the third hour; the sample processed for 3 minutes has titratable acidity of 88.75°T ±3.35 and the control sample - 87.25°T ±3.4. No statistically significant difference can be found between the titratable acidity of the samples with pasteurized milk, both processed and unprocessed by ultrasound.

**Discussion**

In spite of the fact that the results from this experiment have not been combined with an examination of pH and its relation to milk coagulation, we suppose that the results concerning the fermentation process in sheep’s and cow’s milk contradict the ones obtained by Vercet et al. (2002), who established that there was a delay in the coagulation time of the processed milk. Considering the conditions of the experiment conducted by those researchers, the delay may be due to the combined treatment of milk by pressure, temperature and ultrasound. The data from the current experiment correspond to the results obtained by Wu et al. (2002) who think that the ultrasound processing may reduce the relation to milk coagulation, we suppose that the results concerning the fermentation process of acid formation. The lowest titratable acidity after the third hour was measured in the unprocessed raw cow’s milk – 72.5°T ±2.63, which statistically distinguishes it from the sample processed by ultrasound for 3 minutes (80.75°T ±0.48) but not from the sample processed for 1 minute (77.50°T ±0.5). The samples of pasteurized cow’s milk have higher average titratable acidity compared to the samples of raw milk. The pasteurized cow’s milk processed for 1 minute has titratable acidity of 90.25°T ±2.29 after the third hour; the sample processed for 3 minutes has titratable acidity of 88.75°T ±3.35 and the control sample - 87.25°T ±3.4. No statistically significant difference can be found between the titratable acidity of the samples with pasteurized milk, both processed and unprocessed by ultrasound.

In the second hour of incubation, the lowest acidity was measured in the raw sheep’s milk, processed for 3 minutes by ultrasound (Fig. 2). In the control sample, while in the milk processed by ultrasound: in the sample processed for 3 minutes - 33.5°T ±6.2 and in the sample processed for 1 minute - 33.25°T ±1.6, while the measured value in the control sample was 28.50°T ±2.72.

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Based on the data from the current experiment, we agree with Cameron et al. (2009) who state that the ultrasound processing does not have an adverse effect on the fermentation process when using yoghurt cultures. After the third hour of incubation, the measured value of the acidity of sheep’s milk was equal to 0.8-1% of lactic acid and for the cow’s milk the values varied from 0.65 to 0.81%. The obtained data fulfills the requirements for a successfully completed fermentation process because in case there is a release of 1.2-1.4% of lactic acid in milk, it is recommended to start cooling the product in order to prevent the additional release of lactic acid and the deterioration of its organoleptic properties (Duboc and Mollet, 2001).
Figure 1. Changes of the titratable acidity ($\Delta T$) of sheep’s milk during the cultivation of a yoghurt starter culture for 3 hours under a temperature of 43$^\circ$C.

The designation of the samples is as follows: unprocessed raw sheep’s milk (RSM - ●), raw sheep’s milk processed by ultrasound for 1 minute (RSM1 - ▼), unprocessed pasteurized sheep’s milk (PSM - ■), pasteurized milk processed by ultrasound for 1 minute (PSM1 - ♦).

Figure 2. Changes of the titratable acidity ($\Delta T$) of cow’s milk during the cultivation of a yoghurt starter culture for 3 hours under a temperature of 43$^\circ$C.

The designation of the samples is as follows: unprocessed raw cow’s milk (RCM - ●), raw cow’s milk processed by ultrasound for 1 minute (RCM1 - ▼), raw cow’s milk processed by ultrasound for 3 minutes (RCM3 - ■), unprocessed pasteurized cow’s milk (PCM - ♦), pasteurized milk processed by ultrasound for 1 minute (PCM1 - ▲), pasteurized cow’s milk processed by ultrasound for 3 minutes (PCM3 - ●).

Table 1. Compositions of milk samples

<table>
<thead>
<tr>
<th>Milk type</th>
<th>Fat (%)</th>
<th>Solid non fat (%)</th>
<th>Density (g/cm$^3$)</th>
<th>Protein (%)</th>
<th>Titratable acidity ($\Delta T$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>6.43%±0.76</td>
<td>11.05%±0.07</td>
<td>1.0336</td>
<td>5.69%±0.08</td>
<td>23$\Delta T$±1.41</td>
</tr>
<tr>
<td>Cow</td>
<td>4.07%±2.2</td>
<td>8.59%±0.48</td>
<td>1.029</td>
<td>3.33%±0.14</td>
<td>20$\Delta T$±1.41</td>
</tr>
</tbody>
</table>

Conclusions

The ultrasound processing of cow’s and sheep’s milk reduces the coagulation time.

The substitution of thermal pasteurization with ultrasonic treatment provides optimal conditions for the development of lactic acid bacteria.

The ultrasonic treatment has a positive effect on the fermentation process, probably due to the homogenization of milk colloid system under sonication.
References
Chemaf T, Huma Z, Khan MK. 2007. Applications of ultrasound in food technology: processing, preservation and extraction. Ultrasonics Sonochemistry, 18, 813–835.