

Upgrading the technology of functional dairy products by means of fermentation process ultrasonic intensification

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Abstract. Intensification of milk fermentation without negative influence on product quality is a priority research direction in dairy industry. One of the perspective tools for solving this problem is usage of ultrasound. Careful selection of ultrasonic treatment regimens allows to activate lactic-acid bacteria metabolic activity and to improve the efficiency of dairy production. A number of cultivations were carried out for ultrasonic processing effect estimation on *Lactococcus* mixed culture, *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* symbiotic cultures that are often used for dairy fermentation. Milk with added starter culture was treated with ultrasound by means of ultrasonic homogenizer at a frequency of about 30 kHz. Processing duration varied from 1 to 3 minutes and ultrasound power varied from 2 to 8 W. Ultrasonication regimens of fermenting milk allowed accelerating of fermentative process by 10% and improving the quality of final product.

Key words: ultrasound, ultrasonic processing, dried skim milk, reconstitution, intensification, fermentation, *Lactococcus lactis*, *Lactococcus cremoris*, *Lactococcus diacetylactis*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*.

INTRODUCTION

Fermented dairy products are one of the most important sources of essential amino acids, vitamins, trace metals and other biologically active compounds in a human diet maintaining activity of immune system and reducing influence of environmental harmful factors. Nowadays fermented milk is widely used as basis for functional food products.

Fermentation is the longest and one of the most resource-consuming stages during fermented dairy products processing. It demands large floor spaces and high energy consumption for temperature conditions maintenance. Therefore intensification of fermentative processes without negative influence on a final product quality became the priority direction of our research.

Nowadays high consumer demand is observed for natural functional products of high quality which are not containing preservatives and any other chemical additives. So, various non-chemical methods of raw material processing for production improvement become more and more researched (Barba et al., 2012; Chandrasekaran et al., 2013; Kiprushkina & Baranenko, 2014). Ultrasound treatment is one of the most perspective methods for improvement some of the food processes (Rastogi, 2011).

Ultrasound is the sound wave with frequencies above 18–20 kHz, inaudible for human ear. Ultrasonic treatment is often classified by frequency and the energy amount of the generated sound field measured as sound power (W) or sound intensity (W m^{-2}) (McClements, 1995). Most of the sonochemical processes demand application of low-frequency (20–100 kHz) and high-power ultrasound (Chandrapala et al., 2012).

The main effects of high power ultrasound in liquids are the mechanical vibration of medium and moving of the solid particles contained in it; acoustic streaming which can increase mass transfer in medium (Tho et al., 2007) and acoustic cavitation which is one of the most important processes for the ultrasonic treatment (Akopyan & Ershov, 2005).

Ultrasound wave propagate through a medium as a series of compressions and rarefactions. When the rarefaction exceeds the attractive forces between liquid medium molecules, it leads to formation, oscillation and collapse of microbubbles filled with dissolved gases and vapours of this medium. Collapsing bubbles release shock waves (Chisti & Moo-Yong, 1986) which cause intense local heating up to 4,000 K and to increase the pressure up to 1,000 atm (Mason, 1998). Because of that molecules of different compounds dissolved in medium can break apart and form the free radicals which can induce various chemical effects.

When the acoustic energy applied to medium is more than 1 W cm^{-2} , it exceeds the cavitation threshold and formation of gas bubbles becomes continuous (Hmelyov & Popova, 1997). This process refers to transient or stable cavitation (Ashokkumar & Mason, 2007). Cavitation threshold value widely varies depending on the media viscosity and configuration of ultrasonic equipment (Chandrapala et al., 2012) and in the common sonoreactors the ultrasound energy is not exceeded cavitation threshold in most of the reactor volume (Chisti, 2003).

At low frequencies acoustic cavitation can generate very strong physical forces, but the amount of free radicals formed is insignificant (Ashokkumar & Mason, 2007). However, for the dairy processing short time ultrasound treatment is preferred because of pyrolysis reactions inside of the cavitating bubbles. Free radicals induce lipid oxidation that generate various volatile organic compounds in trace amounts and might cause a rubbery flavour and aroma (Riener et al., 2009).

Effects of vibration, acoustic streaming and cavitation induced by ultrasound made it very useful tool for many food production processes. Ultrasound has been used in the food industry since the 1960s for food characterization and cleaning (Mason et al., 1996). Nowadays ultrasonic processing has more applications on dairy factories in such different operations as homogenization, pasteurization, drying and reconstitution of dried milk (Hmelyov & Popova, 1997; Mason, 1998; Villamiel et al., 2000; Ertugay et al., 2004; Makeev et al., 2006; Dolatowski et al., 2007; Dergachyov & Bliadze, 2009; Ashokkumar et al., 2010; Chandrapala et al., 2012).

The most common devices used for the generation of ultrasound are piezoelectric transducers. They change their geometrical sizes under the influence of the alternating high-frequency voltage and convert electrical energy to acoustic energy. Transducers can be mounted directly on the walls of sonochemical reactor (ultra-sonic bath) or can be used as separate submerged device (ultrasonic probe). Ultrasonic baths often used for low power ultrasound processing in order to avoid cavitation damage to the reactor and their sound intensity is highly depends on reactor volume (Mason, 1998). The ultrasonic

probes are preferable for the continuous stable high-power ultrasound processing (Rastogi, 2011).

The most widespread ultrasonic devices in the dairy industry nowadays are ultrasonic homogenizers. They are represented by piezoelectric transducers mounted in a steel pipe. That kind of construction allows applying ultrasound to various purposes at any technological stage of dairy production (Makeev et al., 2006).

It is known that using power ultrasound processing at low frequencies for milk homogenization can increase viscosity and enhance texture characteristics of fermented products (Sfakianakis & Tzia, 2010). It also allows receiving very fine emulsions and altering size distribution of the fat globules in milk by changing ultrasonic power and length of sonication (Zverev & Lobanov, 2005; Ashokkumar et al., 2010). That effect can be useful in combined functional foods production. Another advantage of the ultrasonic homogenisers is the ease of their cleaning relative to traditional homogenizers (Ertugay et al., 2004).

Application of the ultrasonic treatment can also intensify dissolution of dried milk which is very useful and valuable dairy raw material for fermented dairy production. Sonication of dried milk dissolved in water increases its solubility due to breaking of its agglomerates and, thereby, reduces the optimum dissolution temperature to 25°C. Ultrasound treatment also promotes proteins swelling and changes a ratio of free and bound water (Popova, 2013) that is especially important for fermentation process efficiency.

One more positive effect of ultrasound that can be used at dried milk restoration process is foam destruction. It results from gas bubbles pulsation and impact on their surface by the turbulent acoustic streaming (Dergachyov & Bliadze, 2009). The 5 minutes of 20 kHz ultrasound treatment can remove gas bubbles from mixture and prevent the reducing of final product yield and its oxidative degradation (Villamiel et al., 2000).

The low-frequency high-power ultrasound can also cause different effects on metabolic activity of bacteria cells, including changes to organelles within cells, altering of enzyme stability and cell growth properties, breakage of extracellular polymer substances, enhancing mass transport inside and outside of the cell, alteration of cell surface charge and even rupture of cell membranes and cell lysis (Rokhina et al., 2009). For years in food industry high-power ultrasound mostly has been used for cell disruption to release intracellular organelles and enzymes (Chisti & Moo-Yong, 1986; Akopyan & Ershov, 2005) and for pathogens inactivation in food products (Mason et al. 1996; Knorr et al., 2004). In dairy products power ultrasound treatment can be used to inactivate such pathogenic bacteria as *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* (Gera and Doores, 2011; Herceg et al. 2012) in whole and skim milk.

The one of the most perspective and quickly developing directions of sonobiochemistry is application of ultrasound for intensification of cell metabolism and growth (Kwiatkowska et al., 2011). In the liquid media microbial cells always surrounded by liquid film, which can reduce transfer of nutrients and cell by-products (Chisti, 1999). Acoustic streaming, cavitation and other ultrasound effects can thin this film and enhance mass transfer inside and outside of the cells. Thus, controlled ultrasound with suitable sonication regimens which differ for various kinds of

microorganisms can cause beneficial effects on metabolic activity of microbial systems (Chisti, 2003).

High-power ultrasound treatment at low frequencies (about 20–24 kHz) during fermented milk products processing can cause reducing the fermentation time of yogurt (Masuzawa & Ohdaira, 2002), reducing amount of lactose in fermented product (Toba et al., 1990) and increasing the metabolic activity of *Lactobacillus delbrueckii* and various strains of *Bifidobacteria* in milk (Wang & Sakakibara, 1997; Nguyen et al., 2009). Although, higher frequencies application and continuous sonication upon cultivation of *Lactobacillus delbrueckii* causes cells deactivation and intracellular enzymes leakage (Sakakibara et al., 1994; Wang et al., 1996) and can led to longer fermentation process duration (Sfakianakis & Tzia, 2010).

Thus, careful selection of the ultrasonic treatment modes allows achieving activation of lactic-acid bacteria metabolic activity and reduction of fermentation duration. This effect can be used for improving the efficiency of functional dairy production by means of fermentation process acceleration and final product enrichment with native functional compounds produced by microorganisms.

So, ultrasonic processing of fermented mixture after starter culture adding allows combining dried skim milk reconstitution and fermentative process ultrasonic intensification. This can be corresponded with the operating modes of industrial high-power and low-frequency ultrasonic homogenizers and probes which already used on various dairy factories. This technique can allow applying the same equipment for a number of different operations and reducing necessary floor spaces and capital costs, which is especially important for small dairy factories. Besides that, most of the ultrasonic beneficial effects appear over short times and at low frequencies. So, using mid-power short-time ultrasonic treatment also allows minimize some of the ultrasound negative effects and energy costs (Ashokkumar et al., 2010).

A number of cultivations were carried out for estimation an effect of ultrasonic processing on lactic-acid bacteria technological cultures that are often used for milk fermentation. Various milk components such as milk fat and lactose can reduce ultrasound influence on the bacteria cells (Chandrapala et al., 2012). For minimizing the milk components protective effect reconstituted skim milk standardized to lactose amount of 4.5% was used for cultivation.

MATERIALS AND METHODS

Lyophilized mesophilic mixed culture of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, and *Lactococcus lactis* subsp. *cremoris* (biovar *diacetylactis*) applied in production of sour milk and sour cream and thermophilic symbiotic culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* applied for production of yoghurts were used for the study. All cultures were obtained from the All-Russian Research Institute of Fats collection.

High quality dried skim milk (Latvia) was used as a basis for nutrient media. Dried milk was reconstituted to lactose concentration of 4.5% and pasteurized at the temperature of $76 \pm 2^{\circ}\text{C}$ within 20 seconds. Then lyophilized starter cultures were added in quantity of 0.02 g (0.1 activity unit) per liter of reconstituted milk and 22 samples of reconstituted milk with added starter culture were prepared. The volume of each sample was 25 ml.

Laboratory ultrasonic homogenizer SonoPuls mini20 (Bandelin, Germany) with 2.5 mm piezoelectric probe transducer operating at a frequency of about 30 kHz was applied for the ultrasound treatment.

Each sample was treated by ultrasound at the beginning of cultivation and two hours after the beginning of cultivation. Processing time and ultrasound power varied from 1 to 3 minutes and from 2 to 8 W for different samples. The temperature of cultivation was $32 \pm 2^{\circ}\text{C}$ and $40 \pm 2^{\circ}\text{C}$ for the *Lactococcus* mixed culture and for *Streptococcus* and *Lactobacillus* symbiotic culture, respectively.

Titrateable acidity (Turner degrees, $^{\circ}\text{T}$) and pH of the samples were monitored during the cultivation as the key indicators of a product readiness. Cultivation was finished when pH of untreated sample was below 4.4 and the strong casein coagulum was formed. For the *Lactococcus* mixed culture and for *Streptococcus* and *Lactobacillus* symbiotic culture fermentation durations were 9 hours and 6 hours, respectively. Lactose content in the fermented product samples was determined after cultivation for bacteria metabolic activity estimation.

Viscosity depending on shear rate (flow curve) was studied in the final product samples for evaluation of the ultrasound influence on fermented products structure properties (Krus et al., 2000). Flow curves were measured by rotary rheometer RN 4.1 (Rheotest, Germany).

Water activity of reconstituted milk after the first sonication was studied for evaluation of milk proteins condition. High precision dew point water activity meter AVK-4 (SPbSAU, Russia) was used for water activity measurement in the fermented milk samples.

Concentrated sulfuric acid, 5% phenol solution, and 1 M sodium hydroxide solution (Vekton, Russia) were used for the photometric definition of lactose content according to Lawrence (Krus et al., 2000). An optical density of lactose and fermented samples solutions was measured on UV-1800 spectrophotometer (Shimadzu, Japan).

Visual counting of microorganisms and taking the microphotographs of treated and untreated samples were made with Axio Lab.A1 microscope (Carl Zeiss, Germany).

Water activity, titrateable acidity and lactose concentration t-confidence intervals were calculated at the confidence level of 95% based on four measured values for each ultrasonic treatment regimen (Vasilinets & Kolodyaznaya, 2001). All calculations were made in Microsoft Excel.

RESULTS AND DISCUSSION

Cultivation cycle was repeated four times for the purpose of minimizing random deviations (Gvozdev, 2013). After each cultivation cycle the titrateable acidity and lactose content of fermented samples were measured. Average values of fermented samples titrateable acidity and t-confidence intervals are summarized in Tables 1 and 2.

Table 1. Titratable acidity of reconstituted milk samples fermented by *Lactococcus* mixed culture under various ultrasound treatment regimens after 9 hours of cultivation

Ultrasound power, W	Treatment duration, min		
	1	2	3
2	62 ± 1	60 ± 2	63 ± 2
3	62 ± 2	60 ± 2	59 ± 2
4	63 ± 2	64 ± 1	60 ± 2
5	65 ± 2	63 ± 2	62 ± 2
6	69 ± 1	66 ± 1	64 ± 2
7	66 ± 2	61 ± 2	60 ± 2
8	64 ± 2	63 ± 2	58 ± 2
Untreated sample	60 ± 1		

Table 2. Titratable acidity of reconstituted milk samples fermented by symbiotic *Streptococcus* and *Lactobacillus* culture under various ultrasound treatment regimens after 6 hours of cultivation

Ultrasound power, W	Treatment duration, min		
	1	2	3
2	67 ± 2	71 ± 1	68 ± 2
3	68 ± 1	70 ± 2	72 ± 1
4	68 ± 2	70 ± 2	72 ± 2
5	71 ± 2	72 ± 2	73 ± 1
6	66 ± 2	70 ± 2	72 ± 1
7	68 ± 1	71 ± 2	68 ± 2
8	70 ± 2	72 ± 2	72 ± 1
Untreated sample	65 ± 1		

Average values of fermented samples lactose content and t-confidence intervals are summarized in Tables 3 and 4. Water activity, titratable acidity and lactose solution optical density of each sample were defined as average value of three parallel measurements. Titratable acidity in all treated samples was higher and lactose content was lower than in the untreated sample. Obtained results also show that minimum average lactose content correlate with the maximal titratable acidity of fermented media for both cultures.

Table 3. Average lactose content of reconstituted milk samples fermented by *Lactococcus* mixed culture under various ultrasound treatment regimens after 9 hours of cultivation

Ultrasound power, W	Treatment duration, min		
	1	2	3
2	3.32 ± 0.06	3.39 ± 0.06	3.54 ± 0.08
3	3.26 ± 0.05	3.40 ± 0.06	3.68 ± 0.09
4	3.23 ± 0.06	3.30 ± 0.06	3.51 ± 0.05
5	3.16 ± 0.06	3.27 ± 0.08	3.45 ± 0.08
6	3.01 ± 0.06	3.12 ± 0.07	3.47 ± 0.08
7	3.04 ± 0.08	3.09 ± 0.07	3.55 ± 0.09
8	3.08 ± 0.06	3.07 ± 0.09	3.58 ± 0.09
Untreated sample	3.37 ± 0.08		

Table 4. Average lactose content of reconstituted milk samples fermented by symbiotic *Streptococcus* and *Lactobacillus* culture under various ultrasound treatment regimens after 6 hours of cultivation

Ultrasound power, W	Treatment duration, min		
	1	2	3
2	3.37 ± 0.08	2.96 ± 0.05	3.04 ± 0.04
3	3.32 ± 0.06	3.12 ± 0.08	2.96 ± 0.05
4	3.34 ± 0.06	2.93 ± 0.08	2.72 ± 0.05
5	3.31 ± 0.04	2.90 ± 0.05	2.65 ± 0.04
6	3.25 ± 0.05	3.04 ± 0.06	2.79 ± 0.06
7	3.05 ± 0.07	3.00 ± 0.07	2.76 ± 0.06
8	3.02 ± 0.08	2.86 ± 0.06	2.72 ± 0.05
Untreated sample	3.49 ± 0.05		

These results allow suggesting that mid-power short-time 30 kHz ultrasonic treatment can increase primary metabolic activity of both *Lactococcus* mixed culture and *Streptococcus* and *Lactobacillus* symbiotic culture and thus can shorten fermentation time. Visual counting of the microorganisms has shown that for the most part of the ultrasound treated samples overall amount of microbial cells was higher than in the untreated sample. Microphotographs of treated and untreated samples are shown on Figs. 1 and 2.

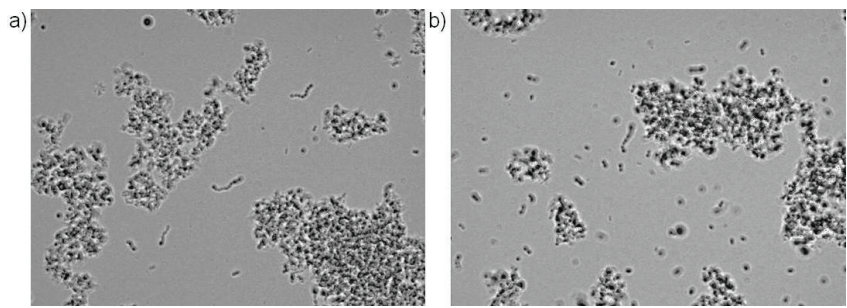


Figure 1. Microphotographs at 900x magnification of *Lactococcus* mixed culture after 9 hours of cultivation: a) untreated sample; b) 6 W ultrasonic treatment within 1 minute.

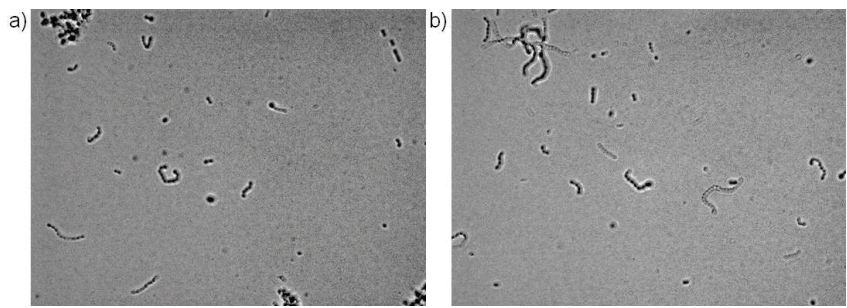


Figure 2. Microphotographs at 900x magnification of symbiotic *Streptococcus* and *Lactobacillus* culture after 6 hours of cultivation: a) untreated sample; b) 5 W ultrasonic treatment within 3 minutes.

It was also noticed that the ratio of various bacteria species also changes with changing of ultrasound treatment power and duration. The tendency for *Streptococcus* to form the longer chains under ultrasound influence was observed (Fig. 2). These effects need further investigation. Water activity of reconstituted milk untreated samples with addition of *Lactococcus* mixed culture and symbiotic *Streptococcus* and *Lactobacillus* culture was 0.9930 ± 0.0003 and 0.9924 ± 0.0003 , respectively. After the first sonication one of the lowest water activity values also were 0.9925 ± 0.0003 under the treatment regimen of 6 W and 1 min for *Lactococcus* and 0.9919 ± 0.0003 under the treatment regimen of 5 W and 3 min for *Streptococcus* and *Lactobacillus* culture. These treatment regimens also showed the minimum lactose content and maximum titratable acidity of fermented samples. This shows maximal intensification of the fermentative processes studied. This effect can be associated with ultrasonic intensification of lactose leaching from the surface of dried milk proteinaceous particles and increase of its availability to microorganisms developed in restored milk (Popova & Potoroko, 2014). Rheological analysis of fermented samples has shown that ultrasound treatment of fermented media at all regimens causes increase of final product viscosity and enhance its thixotropic properties and structure characteristics. These changes were also very significant in the samples treated under the regimens providing fermentation processes highest intensification (Figs. 3, 4).

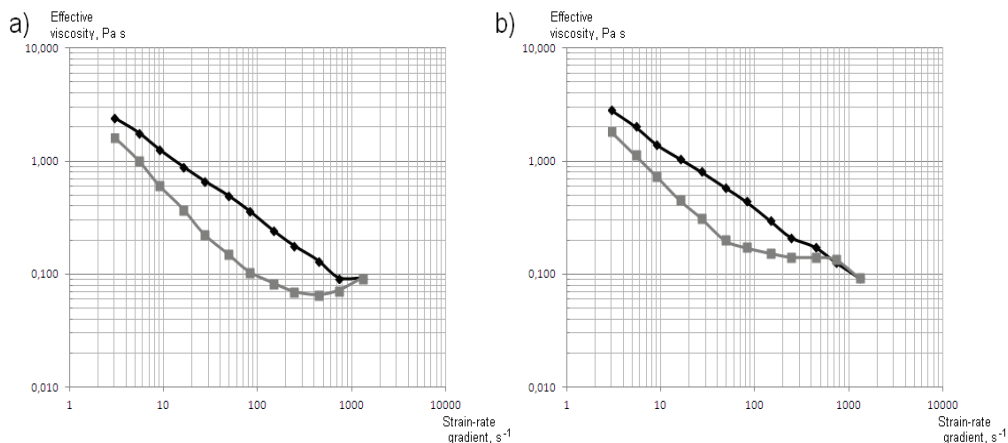


Figure 3. Flow curves of reconstituted milk samples fermented by *Lactococcus* mixed culture after 9 hours of cultivation: a) untreated sample; b) 6 W ultrasonic treatment within 1 minute.

The shelf life of treated samples was also increased. After a week of the samples cold storage at the temperature of 5°C titratable acidity of all samples did not exceed 80 °T and firmness of treated products was proven to be higher than untreated by means of syneresis reduction.

The specific regimens of ultrasound treatment by means of ultrasonic homogenizer were obtained summarizing all data. For the lyophilized mesophilic mixed culture of *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. cremoris*, and *Lactococcus lactis subsp. cremoris* (biovar *diacetylactis*) the regimen of 6 W ultrasonic treatment within 1 minute at the beginning of cultivation and 2 hours after the beginning of cultivation is recommended.

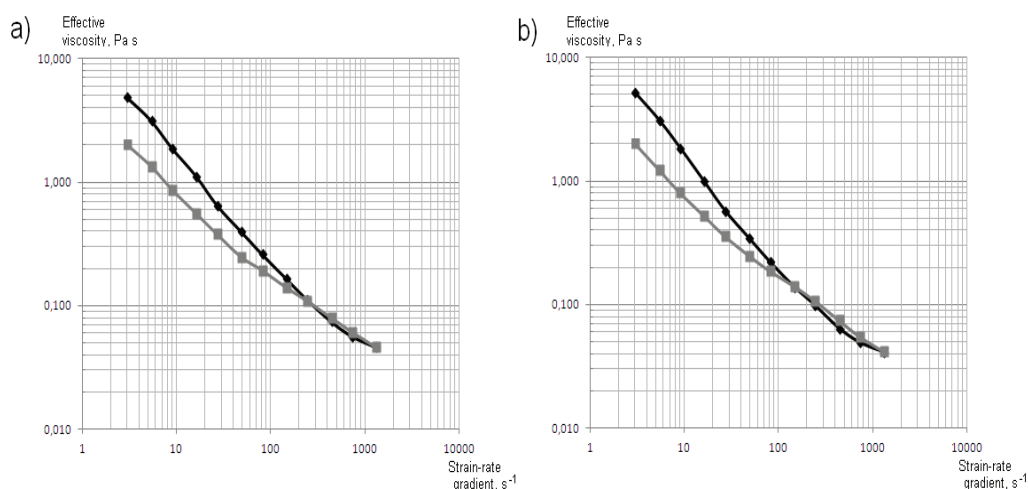


Figure 4. Flow curves of reconstituted milk samples fermented by symbiotic *Streptococcus* and *Lactobacillus* culture after 6 hours of cultivation: a) untreated sample; b) 5 W ultrasonic treatment within 3 minutes.

For the lyophilized thermophilic symbiotic culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* the regimen of 5 W ultrasonic treatment within 3 minute at the beginning of cultivation and 2 hours after the beginning of cultivation is recommended. Increasing of titratable acidity of fermented samples treated by ultrasound on these regimens is shown on Fig. 5.

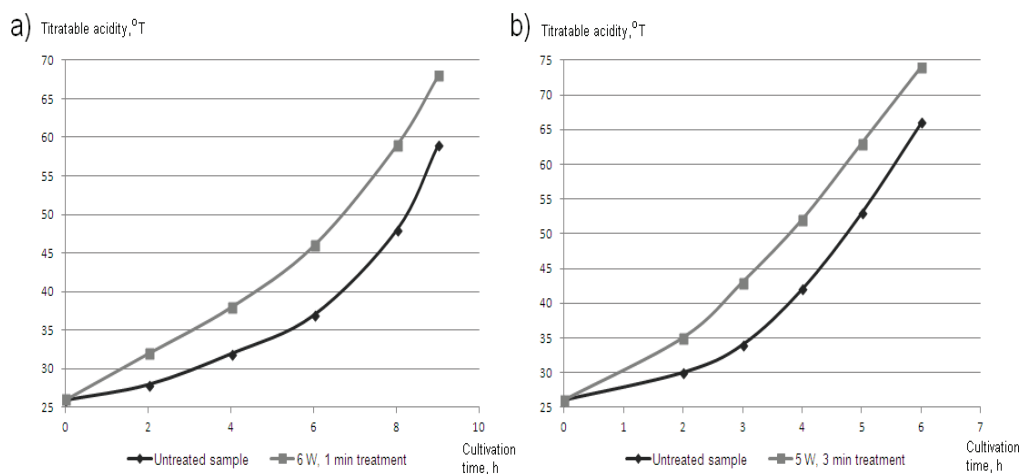


Figure 5. Increasing of titratable acidity in treated and untreated reconstituted milk samples during the fermentation: a) by *Lactococcus* mixed culture; b) by symbiotic *Streptococcus* and *Lactobacillus* culture.

Thus, these regimens provide increasing of the efficiency of fermented dairy production based on reconstituted skim milk by means of reducing the duration of lyophilized microorganisms' revival, accelerating fermentation stage by about 10% and enhancing the texture properties of final products.

CONCLUSIONS

Ultrasonication is a relatively new method in dairy industry and most of the laboratory researches were not approved in industrial scale processes. Meanwhile, ultrasonic equipment can be rarely found on dairy factories, but it is just a matter of time.

Most of developed ultrasound techniques are more safe, energy efficient and economic than their common alternatives (Rastogi, 2011). Due to its high universality such ultrasound techniques can be directly moved from laboratory into fully operational commercial food processes using the industrial ultrasonic equipment providing the required sonication regimens. Ultrasonic equipment can also be adapted to existing processing lines for upgrading different industrial operations (Ashokkumar et al., 2010). It has a good payback on capital investment (Patist & Bates, 2008).

As a result of our research it is possible to make a conclusion that application of fermentation ultrasonic intensification technique in industrial scale will allow reducing production duration and increasing quality of different types of traditional and innovative functional dairy products on the basis of powdered skim milk. Such products can also be naturally enriched with functional substances and have less demand on special additives. This technique can reduce their prime cost and can increase availability of some types of specialised foods for the ordinary consumer. Determined regimens of ultrasonic processing can be applied on some dairy factories that already use ultrasonic homogenizers.

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