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Microwave heat treatment application to pasteurization of human milk

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Abstract

A prototype of microwave pasteurizer has been proposed as an alternative for holder pasteurization (HP) routinely used in Human Milk Bank (HMB), ensuring microbiological safety of human milk (HM). It was shown that the time of heat generation was about 15-16 min shorter by applying the microwave than in HP. Total inactivation of heat-sensitive bacteria *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Staphylococcus epidermidis*, suspended in milk, occurred in the temperature 62-72°C in HP. In the case of heat-resistant enterococci the level of inactivation depended on the conditions of the process and the properties of the strains. The application of microwave heating allows to obtain lower D-value than those achieved during HP. The using of microwave heating at 62.5 or 66°C for 5 or 3 min, respectively, allows to inactivation of HM microbiota. Appropriate microbiological quality of milk is critical for the effectiveness of the pasteurization process.

Industrial relevance: Looking for new methods of donor human milk (HM) preservation is dictated by the necessity of providing microbiological safety and, at the same time, maintain its high nutritional and biological value. The holder pasteurization used in the Human Milk Banks (HMB) (heating at 62°C for 30 min) leads to inactivation of all vegetative forms of microorganisms. Unfortunately, this method causes significant reduction of health benefitting properties of HM. The paper demonstrates the possibility of using the new microwave pasteurizer for preservation of HM, allowing for quick heating of milk to the appropriate temperature and maintaining it in these conditions for a required time. It was shown that the decimal reduction times (D) for strains inoculated to UHT or human milk are several times shorter by using microwave heating than in the commercial pasteurization method. The total inactivation of HM microbiota is obtained after heating at 62.5 and 66°C for 5 and 3 min, respectively.

Keywords: microbiota of human milk; microwave pasteurization; enterococci; decimal reduction time; holder pasteurization

1. Introduction

Breastfeeding is unquestionably the best way of feeding newborns and infants. Composition of HM is perfectly adapted in quality and quantity of compounds to the needs of developing children at every stage of their growth. Breast milk provides not only the basic nutrients but also is the source of biological components increasing the child's immunity against bacteria and viruses. A newborn does not yet have a mature immune system, so this first food supplements these deficiencies.

There are situations when a baby cannot be directly fed with its mother's milk. This regards to especially premature infants, fed in the initial period only intravenously, and children with extremely low birth weight or health problems. Then it is essential to collect and appropriately preserve the breast milk, so that a baby can be fed with it in the chronological order. This task is carried out at the HMB. HM is not a sterile product and contains microorganisms, which are commonly found in mammary glands and on the skin of the mother. Among them sometimes are pathogenic species such as, for example, *S. aureus.* Inappropriate handling of milk can also lead to cross-contamination. In many studies the incidental presence of microorganisms in milk, such as *Escherichia coli*, *Klebsiella* sp. (Eja, Asikong, Udo, Mboto, & Arikpo, 2006), *Acinetobacter* (*Acinetobacter baumanni*, *Acinetobacter lwoffii*, *Acinetobacter haemoliticus*), *Citrobacter freundii*, *Serratia liquefaciens* and aerobic gram-positive bacilli (Kamianowska, Szczepański, Bebko, Kamianowski, & Milewski, 2008), *Listeria monocytogenes* (Svabic-Vlahovic, Pantic, Pavicic, & Bryner, 1988) or rickettsia *Coxiella burnetii* (Kumar, Yadav, & Kakkar, 1981) has been noted. Milk intended for the beneficiaries of HMB should not contain any pathogens and viruses. For this reason, milk in HMB is usually pasteurized at 62.5°C for 30 min (it is so-called holder pasteurization). Heating in these conditions leads to eliminating potentially dangerous microorganisms (viruses and pathogenic vegetative bacteria) from HM. It is carried out in special pasteurizers that make it possible to control the pasteurization process and rapid cooling of the milk to 4°C. The conditions (temperature, time) of holder pasteurization were adopted on the basis of researches conducted at the turn of the XIX/XX century. Based on these studies, in 1924, Public Health Reports defined pasteurization as a heating process at temperature not less than 61.1°C by 30 min (Holsinger, Rajkowski, & Stabel, 1997) and was called Low-Temperature-Long-Time (LTLT) pasteurization. This method allows for the effective elimination of most vegetative pathogenic microorganisms, however, leads to a significant decrease of the content of many nutrients and bioactive milk components. Therefore, to guarantee microbiological safety and simultaneously to maintain the high nutritional and biological value of HM, new techniques of preservation are searched for. An alternative method may be the use of High-Temperature-Short-Time (HTST) pasteurization. The possibility of using HTST pasteurization, which would allow for better preservation of biochemical properties of HM than LTLT pasteurization, is rarely tested because of the lack of the necessary equipment for such processing of small amounts of HM (Jensen & Jensen, 1992). The use of HTST rather than LTLT pasteurization is dictated by the principle, which says that the microorganisms are destroyed faster than nutrients during rising of the temperature of pasteurization. This phenomenon is used in food processing, where the goal is to replace the heating at lower lethal temperatures in a longer time with heating at high temperatures and in a shorter time to achieve the same biological effect. This procedure contributes to the protection of nutrients during the heat pasteurization process.

The aim of these studies was determination of survival of selected bacterial strains inoculated into cow milk – (UHT, 3.2% of fat) or into sterilized HM (as a model condition), and microbiota of HM, after microwave HTST pasteurization at different temperatures and comparison with traditional HP. A specially designed microwave pasteurizer (Enbio Technology – prototype) was used in these studies. This device differs from common microwave ovens because it allows for pasteurization of small volumes of milk in strictly programmed time (several seconds) at a given temperature. This device has the function of rapid cooling of milk and self-cleaning option. In addition, all experiments were carried out under conditions that can be applied at HMB.

2. Material and methods

2.1. Materials

UHT milk with a fat content of 3.2% from one manufacturer was purchased from a local market. Mature milk was collected from healthy mothers who gave birth on the scheduled date and without complications at the Department of Obstetrics of the Clinical Hospital in Gdańsk. All newborns were in good health (Apgar score of 9 - 10) with normal birth weight (3100 ÷ 3800 g). The collected milk was pooled, divided into 50 mL samples and heated at different conditions.

All of the experimental procedures were approved by the Local Ethics Committee of the Medical University of Gdansk. The patients gave written consent to participate in the study.

2.2. Cultures and growth conditions

The following bacterial strains were used: *Escherichia coli* K-12 PCM2560 (NCTC 10538), *Pseudomonas aeruginosa* PCM499, *Staphylococcus aureus* PCM 2054 (ATCC 25923), *Staphylococcus epidermidis* PCM 2118, *Enterococcus faecalis*

PCM896 and PCM1861, as well as *Enterococcus hirae* PCM2559 and *Enterococcus durans* PCM1857 from the Polish Collection of Microorganisms, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences, Wrocław, Poland.

The cultures in stationary phase were prepared by inoculating 100 mL of TSB (tryptic soy broth) with 100 μL of liquid culture (at stationary phase of growth) and incubating it at 37 °C for 24 h with shaking.

2.3. Preparation of cell suspensions

The cells in the stationary phase of growth were resuspended in UHT milk or in HM, previously sterilized at 121°C for 20 min, to give viable counts of about 10^5 CFU/mL of the final concentration. The size of the inoculum has been determined on the basis of the maximum microbial contamination of human milk that is accepted in HMB (Arslanoglu et al., 2010; Malinowska-Pańczyk & Rosiak, 2017).

2.4. Microwave heating

The samples of milk (50 mL) in breastmilk bottles (Medela Ltd.) were placed into the chamber of microwave pasteurizer. The milk was pasteurized using the prototype EnbioJet Microwave Flow Pasteurizer (Enbio Technology Co., Kosakowo, Poland) dedicated to small volume of liquid products (Patent Application no. PL 384854). This equipment allows on quick heating of a small volume of milk, and rigorous control of temperature and process time. The samples were heated at temperature from the range of 62.5 - 72°C for 0, 1, 3, 5 and 10 min, and then were automatically cooled to about 15°C. Triplicate determinations were made for each time and temperature.

2.5. Holder pasteurization

Milk was pasteurized following the procedure of the Human Milk Banking. The samples (50 mL) in breastmilk bottles (Medela Ltd.) were placed in a water bath, heated to 62.5-72°C and keep at this temperature for 0 (immediately after reaching the set temperature), 10, 20 and 30 min. The temperature of milk during heat processing was monitored using a calibrated thermometer placed into control bottle, containing the same volume of non-contaminated milk, and heated in a water bath with all the other samples. After the heat processing, the milk was rapidly cooled in an ice bath and stored prior to determination of viable counts. Untreated samples were used as control.

2.6. Enumeration of viable cells

The control (unpasteurized) and heat treated milk were serially diluted with buffered saline peptone water (pH 7.0). Dilutions of the milk samples were plated on appropriate media and the plates were incubated for 48 h at 37°C. The media and growth conditions of microbiota of human milk are presented in the Table 1. The media were purchased from Merck KGaA.

2.7 Statistical analysis

The data presented in the figures and table are average values of at least three replications with standard deviation. Analysis of variance (one-way procedure) was performed to evaluate differences between treatments using the Statistica 8.0.

3. Results and discussion

3.1. Effect of heating on bacteria inoculated in milk

Depending on the method used to generation of heat, the time needed to reach the temperature in the range of 62.5 - 72°C was different. In the case of HP it was about 18 min. During microwave-induced heating the required temperature was achieved in 1.4 min or 3 min for 62.5°C and 66-72°C, respectively (Table 2).

The results shown in a Table 3, indicate, that the gram-negative bacteria *E. coli* and *P. aeruginosa* and gram-positive bacteria *S. aureus* and *S. epidermidis* have been completely inactivated already at the time of reaching the temperature in the range 62.5-72°C during HP. Czank, Prime, Hartmann, Simmer, and Hartmann, (2009) showed that holding at 62.5°C caused lowering the number of *E. coli*, *S. epidermidis* and *S. aureus* by 1 log cycle after 5.4 min, 5.6 min and 11.9 min, respectively. These differences between our data and those of Czank et al. (2009) may results from variation in heat-resistance of particular strains belonging to the same species as well as from the method of heating of samples (especially from the sample size). In the case of thermoresistant strains *Enterococcus* sp., the populations decreased as the temperature increased and only after the time of reaching the temperature 72°C all enterococci strains were not detected in 1 mL of sample (Table 3).

During microwave heating, when the required temperature was achieved, the number of microorganisms decreased depending on the thermal sensitivity of the tested strains. Total inactivation of all thermosensitive strain (*E. coli, P. aeruginosa, S. aureus, S. epidermidis)* and *E. faecalis* PCM896 were observed immediately after reaching 72°C. The time needed to reach the required temperatures exerts important effect on the survival fraction of tested microorganisms inoculated in milk.

The survival of microorganisms during heating decreased linearly with time, indicating a first order kinetics. To compare the effectiveness of the heating methods and optimize the pasteurization process conditions, the calculation of inactivation rate (k) and decimal reduction times (D_T-value) was carried out for the strains surviving at the time of achieving the required temperature, according to equations:

$$D_T = t / \log_{10} (N_0 / N_t)$$

$$k = 2,3026 / D_T$$

where: N_0 - the initial cell count, N_t - the number of cells after time t of heating at temperature T.

Table 4 shows D_T-values and k parameters for thermosensitive bacteria heated using microwave fields. Decreasing the population of these bacteria by one log cycle was possible after less than 0.5 min. At temperature 62.5°C the most resistant was *E. coli* strain because the $D_{62.5°C}$ for this strain was the longest and the rate of inactivation was 6.4 min^{-1}. At higher temperatures the differences between the strains were statistically insignificant.

In the case of thermoresistant enterococci heated by HP, the $D_{62.5°C}$ amounted to 5.67 and 33.2 min for *E. hirae* and *E. durans*, respectively. The number of cells in population of *E. faecalis* strains decreased only slightly after 30 min treatment in this temperature, therefore, the calculated $D_{62.5°C}$ were very high and reached 66.2 min and 71.1 min for *E. faecalis* PCM896 and *E. faecalis* PCM1861, respectively. The value of the inactivation rates $k_{62.5°C}$ was very low for all enterococcus strains. Bacteria belonging to *Enterococcus* sp. are considered, as the most heat-resistant among non-spore forming bacteria (Garg & Mital, 1991; Perez, Lorenzo, Garcia, Hernandez, & Ordonez, 1982) and showed only about 0.5 log cycle loss of viability after heating in neutral environment at 60°C by 30 min (Clark, Witter, & Ordal, 1968). At 66°C, the most heat resistant was *E. faecalis* PCM896. Heating for 12 min is needed to reduce the population of this strain by one log cycle at 66°C. The most sensitive was *E. hirae* with $D_{66°C}$ = 0.1 min. Strains *E. faecalis* PCM896, *E. hirae* and *E. durans* were not detected already after reaching of temperature 70 and 72°C and it was impossible to calculate the D-values. Only the strain *E. faecalis* PCM1861 survived the time to reach temperature of 70°C and $D_{70°C}$ amounted to 0.95 min (Table 3). In turn, Perez et al., (1982) have shown that heating at 64°C results in the inactivation of 90% of the population of *E. durans*, *E. faecium* and *E. faecalis* suspended in cow's milk after 13.4, 6.3 and 4.5 min respectively. Similarly to our results, with increasing temperature, the D-values of these strains were shorter. Heating at 72°C led to shortening of the D time to a few seconds. Many factors influence the thermal resistance of enterococci, such as time-temperature combinations and properties of particular strains. It is known that the differences in heat sensitivity can appear not only between species but also among strains within one species and can result from various *k* and D_T-values.

Application of microwave heating allows to obtain lower D-value for enterococci than those achieved during HP. This parameter estimated for these bacteria, at all temperatures, was in the range 1.5 - 4 min depending on properties of the strain and temperature used, except $D_{62.5°C}$ calculated for *E. faecalis* PCM1861and *E. durans.*

The available literature data regarding the effect of microwave heating on survival of microorganisms are difficult to compare, because the process parameters are often described only by the power unit or the frequency of electromagnetic waves in microwave oven without temperature value. Górecka, Grochowska, Windyga, Ścieżyńska, and Karłowski, (1999) showed that microwave assisted heating (600 W) for 6 min caused complete inactivation of a strain belonging to *Enterococcus* sp. (initial number 10^7 CFU/mL). On the other hand microwave heating with a frequency of 2450 MHz with a power of 1500 W for 30 min on *E. faecalis* (initial number of cells was 10^9 per mL) reduced the population only by 1 log cycle (Lechowich, Beuchat, Fox, & Webster, 1969). These discrepancies are probably due to different heat resistance of the strains belonging to this species or initial population size. It is known that the lethal effect of temperature is also dependent on level of initial contamination.

In the literature there are few data about heat-sensitivity of enterococci in HM (introduced to HM or microbiota). It has been checked whether the same time of heating is sufficient to achieve the total inactivation if the bacteria were suspended in HM instead of UHT milk. In both, UHT milk and HM, complete inactivation of bacteria occurred after the same time of HP (data not shown). In the case of microwave heating enterococci suspended in HM were more resistant than in UHT milk. To achieve total inactivation, longer heating times were needed in some cases (Table 6). As was reviewed by Andreas, Kampmann, and Mehring Le-Doare (2015) and Lis, Orczyk-Pawiłowicz, and Kątnik-Prastowska (2013) the composition of cow milk differ from HM. Especially a content of carbohydrates can affect the protective effect. HM contains 70 g/L of lactose and 7 g/L of oligosaccharides, whereas UHT milk only 48 g/L and 0,1 g/L, respectively.

3.2. Effect of heating method on microbiota of breast milk

It was demonstrated that the time needed to achieve complete inactivation of microbiota depends on the process temperature and initial microbial contamination of milk. Figures 1, 2, 3 and 4 show survival of natural and cross-contaminated microbiota of HM (TBC, LAB, enterococci, coagulase-positive staphylococci and coli group bacteria). HP at 62.5°C for 30 min did not allow a complete reduction all groups of microorganisms when the initial TBC was above the maximum permissible level (>10^5 CFU/mL). After this time lactic acid bacteria (LAB) were detected in milk (about 1 log CFU/mL) (Fig. 1B). The use of microwave heating at this temperature for the pasteurization of HM with high initial contamination also did not allow to achieve the total pasteurization effect even after 10 min of the process (Fig. 2C). The efficiency of pasteurization at 62.5°C was higher when the initial contamination of milk was lower (Fig. 1A, 2A and B). Bacteria were not detected in 1 mL of milk after reaching 62.5°C during HP or after 3 or 5 min of microwave assisted heating.

Increasing the temperature to 66°C allowed to reduce the heating time needed to completely inactivate of the bacteria compared to the effect of 62.5°C. The population of HM microbiota did not survive just after reaching the set temperature (HP) when its number did not exceed 10^5 CFU/mL (TBC) (Fig. 3A). While the initial contamination was greater than 10^5 CFU/mL, the same level of inactivation was possible after 30 min of heating at this temperature (Fig. 3B). Microwave heating at 66°C caused total pasteurization effect after 1 or 3 min of heating depending on the initial level of TBC (Fig. 4).

Coliform bacteria (similar to *E. coli* at model conditions) were sensitive to HP conditions and were not detected after reaching of temperature 62.5°C and 66°C (Fig. 1 and 3). Microwave pasteurization inactivated the coliforms after 3 min of heating at 62.5°C or after reaching of 66°C (Fig. 2 and 4). It was a time shorter than the one for *E. coli* in model conditions. In the case of coagulase-positive staphylococci when the number of cells was about 10^3 CFU/mL, HP and microwave heating caused total inactivation after reaching the temperature 62.5 and 66°C (Fig 1A, 2, 3 and 4). A longer time (30 min at 62.5°C) was needed to inactivate all coagulase-positive staphylococci when their number in raw milk was about 10^5 CFU/mL (Fig. 1B).

The number of enterococci in most milk samples ranged from 0-10^3 CFU/mL, except for the sample heated during HP at 62.5°C (Fig. 1B). This group of bacteria was sensitive to heating at 62.5 and 66°C and was not detected usually after reaching the required temperature (Fig 1A and 3A, B). The longest time needed to complete inactivation of enterococci by microwave heating was 3 min (Fig. 2 and 4). In recent years there have been reports that bacteria from *Enterococcus* genus can be opportunistic pathogens and cause infection especially of hospitalized, immunocompromised patients. Among the species, which caused the disease are mentioned strains of *E. faecalis* and less *E. faecium*. They have the ability of transferring virulence factors and antibiotics resistance into closely related strains or cells of other species of bacteria. However, it has been shown, that strains isolated from HM do not possess the characteristic features of pathogenic strains (Reviriego et al., 2005; Togay, Temiz, Çelebi, Acik, & Yalcin, 2014). On the other hand, these bacteria are commonly found in the environment and in the intestinal tract of healthy humans and animals, as well as in the HM. Some strains belonging to *Enterococcus* genus are used as probiotic, which exert positive impact on intestinal tract of humans. Enterococci also produce natural antimicrobial substances with broad-spectrum inhibiting the growth of pathogenic microorganisms. Both, European and American organizations responsible for food safety did not regulate permissible, acceptable levels of these microorganisms in food products, as well as in breast milk. Due to the lack of virulence among strains found in breast milk, a small number of the population of these bacteria after pasteurization should not raise objections.

4. Conclusions

In the last 50 years there have been several reports in the literature regarding the possibility of using the HTST method to preserve HM (Dhar, Fichtali, Skura, Nakai, & Davidson, 1996; Giribaldi et al., 2016; Goldblum et al., 1984; Klotz et al., 2017). However, it is difficult to compare the obtained results due to the many differences in the conditions used: temperature, heating time, sample size, challenging test used to determine the effectiveness of pasteurization. Some of the device prototypes used need to be adapted so that they can be routinely used in HMB. In our work we showed that total pasteurization can be achieved in shorter time than by using the holder method. The using of microwave heating allows to inactivate of all bacterial strains inoculated to human milk and its microbiota. The factor determining the effectiveness of the pasteurization process is the appropriate microbiological quality of milk. The contamination of milk with microorganisms above 10^5 CFU/mL may cause that even 30 min heating at 62.5°C will not effectively eliminate all bacteria in milk.

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References

Andreas, N. J., Kampmann, B., & Mehring Le-Doare, K. (2015). Human breast milk: A review on its composition and bioactivity. *Early Human Development*, *91*(11), 629–635. https://doi.org/10.1016/j.earlhumdev.2015.08.013

Arslanoglu, S., Bertino, E., Tonetto, P., De Nisi, G., Ambruzzi, A. M., Biasini, A., … Moro, G. E. (2010). Guidelines for the establishment and operation of a donor human milk bank. *The Journal of Maternal-Fetal and Neonatal Medicine*, *23*, 1–20.

Clark, C. W., Witter, L. D., & Ordal, Z. J. (1968). Thermal injury and recovery of *Streptococcus faecalis*, *16*(11), 1764–1769.

Czank, C., Prime, D. K., Hartmann, B., Simmer, K., & Hartmann, P. E. (2009). Retention of the immunological proteins of pasteurised human milk in relation to pasteurizer deisgn and practise. *Pediatirc Research*, *66*(4), 374–379.

Dhar, J., Fichtali, J., Skura, B. J., Nakai, S., & Davidson, A. G. F. (1996). Pasteurization efficiency of a HTST system for human milk. *Journal of Food Science*, *61*(3), 569–573. https://doi.org/10.1111/j.1365-2621.1996.tb13160.x

Eja, M. E., Asikong, B. E., Udo, S. M., Mboto, C., & Arikpo, G. E. (2006). Microbiological and biochemica lassessment of the surface area of breast nipples and breast milk of lactating women in calabar south eastern Nigeria. *The Southeast Asian Journal of Tropical Medicine and Public Health*, *37*, 215–221.

Garg, S. K., & Mital, B. K. (1991). Enterococci in milk and milk products. *Critical Reviews in Microbiology*, *18*, 15–45. https://doi.org/10.3109/10408419109113508

Giribaldi, M., Coscia, A., Peila, C., Antoniazzi, S., Lamberti, C., Ortoffi, M., … Cavallarin, L. (2016). Pasteurization of human milk by a benchtop High-Temperature Short-Time device. *Innovative Food Science and Emerging Technologies*, *36*, 228–233. https://doi.org/10.1016/j.ifset.2016.07.004

Goldblum, R. M., Dill, C. W., Albrecht, T. B., Alford, E. S., Garza, C., & Goldman, A. S. (1984). Rapid high-temperature treatment of human milk. *The Journal of Pediatrics*, *104*(3), 380–385. https://doi.org/10.1016/S0022-3476(84)81099-9

Górecka, K., Grochowska, A., Windyga, B., Ścieżyńska, H., & Karłowski, K. (1999). The effect of microwave irradiation on survivability of pathogens. (in Polish) *Http://Www.Pzh.Gov.Pl/Oldpage/Biblioteka/Badania/1999/19_8.Html.*

Holsinger, V. H., Rajkowski, K. T., & Stabel, J. (1997). Milk pasteurisation and safety: a brief historyand update. *Revue Scientifique et Technique (International Office of Epizootics)*, *16*, 441–451.

Jensen, R. G., & Jensen, G. L. (1992). Specialty lipids for infant nutrition. I. Milks and formulas. *Journal of Pediatric Gastroenterology and Nutrition*, *15*, 232–245.

Kamianowska, M., Szczepański, M., Bebko, B., Kamianowski, G., & Milewski, R. (2008). Analiza bakteriologiczna pokarmu kobiecego, uzyskanego w warunkach szpitalnych i domowych od matek noworodków z małą urodzeniową masą ciała.

Przegląd Pediatryczny, *38*, 291–297.

Klotz, D., Joellenbeck, M., Winkler, K., Kunze, M., Huzly, D., & Hentschel, R. (2017). High-temperature short-time pasteurisation of human breastmilk is efficient in retaining protein and reducing the bacterial count. *Acta Paediatrica, International Journal of Paediatrics*, *106*(5), 763–767. https://doi.org/10.1111/apa.13768

Kumar, A., Yadav, M. P., & Kakkar, S. (1981). Human milk as a source of Q-fever infection in breast-fed babies. *Indian Journal of Medical Research*, *73*, 510–512.

Lechowich, R. V, Beuchat, L. R., Fox, K. I., & Webster, F. H. (1969). Procedure for evaluating the effects of 2,450-Megahertz microwaves upon *Streptococcus faecalis* and *Saccharomyces cerevisiae*. *Applied Microbiology*, *17*, 106–110.

Lis, J., Orczyk-Pawiłowicz, M., & Kątnik-Prastowska, I. (2013). Promotion of breastfeeding in Europe: Pilot testing the blueprint for action' project. Protection, promotion and support of breast-feeding in Europe: progress from 2002 to 2007. *Postępy Higieny i Medycyny Doświadczalnej*, *67*, 529–547.

Malinowska-Pańczyk, E., & Rosiak, E. (2017). Bezpieczeństwo postępowania z mlekiem w banku mleka. Potencjalne zagrożenia związane z mlekiem kobiecym z banku mleka. In A. Wesołowska (Ed.), *Banki mleka w Polsce. Funkcjonowanie w podmiotach leczniczych - idea i praktyka* (pp. 149–155). Warszawa, Bank Mleka Kobiecego.

Perez, B. S., Lorenzo, P. L., Garcia, M. L., Hernandez, P. E., & Ordonez, J. A. (1982). Heat resistance of enterococci. *Milchwissenschaft*, *37*, 724–726.

Reviriego, C., Eaton, T., Martin, R., Jimenez, E., Fernandez, L., Gasson, M. J., & Rodriguez, J. M. (2005). Screening of virulence determinants in *Enterococcus faecium* strains isolated from breast milk. *Journal of Human Lactation*, *21*, 131–

137.

Svabic-Vlahovic, M., Pantic, D., Pavicic, M., & Bryner, J. H. (1988). Transmissin of *Listeria monocytogenes* from mother's milk to her baby and to puppies. *Lancet*, *19*, 1201.

Togay, S. Ö., Temiz, A., Çelebi, A., Acik, L., & Yalcin, S. S. (2014). Investigation of potential virulence genes and antibiotic resistance characteristics of *Enterococcus faecalis* isolates from human milk and colostrum samples. *Turkish Journal of Biology*, *38*, 357–364.

Figure captions

Figure 1.

The effect of HP at 62,5°C on viability of microbiota of HM; (■) TBC; (□) LAB; (▨) enterococci; (▤) coli group; (⊞) coagulase-positive staphylococci;

A and B mean samples with different initial contamination

Figure 2.

The effect of microwave heating at 62,5°C on viability of microbiota of HM (■) TBC; (□) LAB; (▨) enterococci; (▤) coli group; (⊞) coagulase-positive staphylococci;

A, B and C mean samples with different initial contamination

Figure 3.

The survival of microbiota of HM during HP at 66°C; (■) TBC; (□) LAB; (▨) enterococci; (▤) coli group; (⊞) coagulase-positive staphylococci;

A and B mean samples with different initial contamination

Figure 4.

The survival of microbiota of HM during microwave heating at 66°C; (■) TBC; (□) LAB; (▨) enterococci; (▤) coli group; (⊞) coagulase-positive staphylococci;

A and B mean samples with different initial contamination

Table 1. Media and growth conditions

	Medium	Conditions
Total Bacterial Count (TBC)	Plate count agar	30ºC for 48 h
Lactic Acid Bacteria (LAB)	MRS agar	
Coliform bacteria	Chromocult® Coliform agar	
Enterococcus sp.	Chromocult® Enterococci agar	
Coagulase-positive *Staphylococcus* sp.	Baird-Parker agar	37ºC for 48 h
Strains suspended in UHT and HM	Tryptic soy agar (TSA)	

Table 2. Average time of reaching the set temperature depending on the method of heating

Method of heating	Time of reaching of temperature [min]			
	62,5°C	66°C	70°C	72°C
HP	18±0.8^{a,b,A}	17±1.0^{a,A}	18±1.2^{a,b,A}	19±0.9^{b,A}
Microwave	1.4±0.3^{a,C}	3±0.5^{b,C}	3±0.5^{b,B}	3±0.5^{b,C}

$_{a-b}$ values for a particular column followed by different letters differ significantly (p<0.05)
$_{A-C}$ values for a particular row followed by different letters differ significantly (p<0.05)
(mean ± SD, n = 3)

Table 3. The effect of temperature reaching time on the reduction (N_r/N_0) of population of bacteria inoculated into cow milk

Strain	Temperature [°C]	HP	Microwave heating
		Log N_r/N_0	
E. coli K-12	62.5	5.9±0.21*	0.3±0.15
	66.0	5.9±0.13*	3.8±0.17
	70.0	5.9±0.15*	3.7±0.30
	72.0	5.9±0.19*	5.4±0.21*
P. aeruginosa PCM499	62.5	5.8±0.10*	1.9±0.24
	66.0	5.8±0.05*	4.6±0.11
	70.0	5.8±0.3*	4.3±0.20
	72.0	5.8±0.25*	4.8±0.13*
S. epidermidis PCM 2118	62.5	5.5±0.18*	3.3±0.25
	66.0	5.5±0.14*	3.4±0.07
	70.0	5.5±0.23*	3.5±0.18
	72.0	5.5±0.15*	5.0±0.13*
S. aureus PCM 2054	62.5	5.2±0.15*	0.9±0.18
	66.0	5.2±0.20*	3.2±0.02
	70.0	5.2±0.05*	3.5±0.10
	72.0	5.2±0.04*	4.8±0.05*
E. faecalis PCM896	62.5	<0.2	<0.2
	66.0	0.5±0.05	-
	70.0	5.2±0.20*	1.0±0.02
	72.0	5.2±0.32*	1.4±0.05
E. faecalis PCM1861	62.5	<0.2	<0.2
	66.0	0.7±0.21	3.6±0.30
	70.0	4.8±0.20	3.7±0.20
	72.0	5.3±0.30*	4.9±0.06*
E. hirae PCM2559	62.5	0.62±0.03	<0.2
	66.0	3.8±0.11	0.5±0.02
	70.0	5.2±0.40	0.5±0.03
	72.0	5.4±0.10*	0.6±0.02
E. durans PCM1857	62.5	<0.2	<0.2
	66.0	0.5±0.16	1.1±0.18
	70.0	5.4±0.24*	1.8±0.06
	72.0	5.4±0.06*	2.6±0.2

N_r – bacterial count [CFU/mL] after reaching time for required temperature; N_0 – bacterial initial population [CFU/mL]; * – total inactivation of bacterial population; (mean ± SD, n = 3)

Table 4. Inactivation rates (*k*) and decimal reduction time (D_T-value) of selected thermosensitive bacteria suspended in cow milk during microwave pasteurization

Species	Temperature [°C]	Microwave heating	
		k [min^{-1}]	D_T [min]
E. coli K-12	62.5	6.40	0.36
	66	15.35	0.15
	70	46.05	0.05
	72	-	0.003
P. aeruginosa PCM499	62.5	7.94	0.29
	66	16.44	0.14
	70	46.05	0.05
	72	-	0.003
S. epidermidis PCM 2118	62.5	12.79	0.18
	66	14.40	0.16
	70	46.05	0.05
	72	-	0.003
S. aureus PCM 2054	62.5	13.54	0.17
	66	23.03	0.10
	70	46.05	0.05
	72	-	0.003

Table 5. Effect of heating method on the inactivation rates (*k*) and decimal reduction time (D_T-value) of selected enterococci suspended in cow milk during holder and microwave pasteurization

Species	Temperature [°C]	HP		Microwave heating	
		k [min^{-1}]	D_T [min]	*k* [min^{-1}]	D_T [min]
E. faecalis PCM896	62.5	0.035	66.2	0.17	13.7
	66	0.19	12.1	0.61	3.8
	70	-	N.d.	1.32	1.75
	72	-	N.d.	1.50	1.54
E. faecalis PCM1861	62.5	0.032	71.1	1.54	1.5
	66	0.352	6.53	2.56	0.9
	70	2.423	0.95	2.65	0.87
	72	-	N.d.	3.65	0.63
E. hirae PCM2559	62.5	0.406	5.67	0.57	4.07
	66	23.02	0.1	0.89	2.6
	70	-	N.d.	1.35	1.7
	72	-	N.d.	1.54	1.5
E. durans PCM1857	62.5	0.07	33.2	0.16	14.7
	66	0.51	4.5	1.15	2.0
	70	-	N.d.	1.35	1.7
	72	-	N.d.	1.53	1.5

N.d. – not determined, inactivation took place during reaching of required temperature

Table 6. Heating time needed to achieve complete inactivation of selected bacteria inoculated into HM

Strain	Heating time [min] at temperature							
	62.5°C		66°C		70°C		72°C	
	UHT	HM	UHT	HM	UHT	HM	UHT	HM
E. coli (5.1±0.06)*	5	10	5	10	1	3	TRT	TRT
P. aeruginosa (5.0±0.03)*	5	5	3	3	1	3	TRT	TRT
S. epidermidis (5.2±0.05)*	5	5	3	5	1	3	TRT	TRT
S. aureus (4.7±0.12)*	5	5	1	3	1	3	TRT	TRT
E. faecalis PCM 896 (5.1±0.01)*	-[1]	-[1]	10	-[1]	10	-[1]	10	10
E. faecalis PCM1861 (4.7±0.60)*	10	-[1]	10	-[1]	3	10	3	3
E. hirae (4.6±0.08)*	-[1]	-[1]	-	-[1]	5	5	1	5
E. durans (4.4±0.01)*	-[1]	-[1]	10	-[1]	3	3	5	5

TRT – temperature reaching time;
* - log CFU/mL in control sample; [1] – no total inactivation in experimental conditions

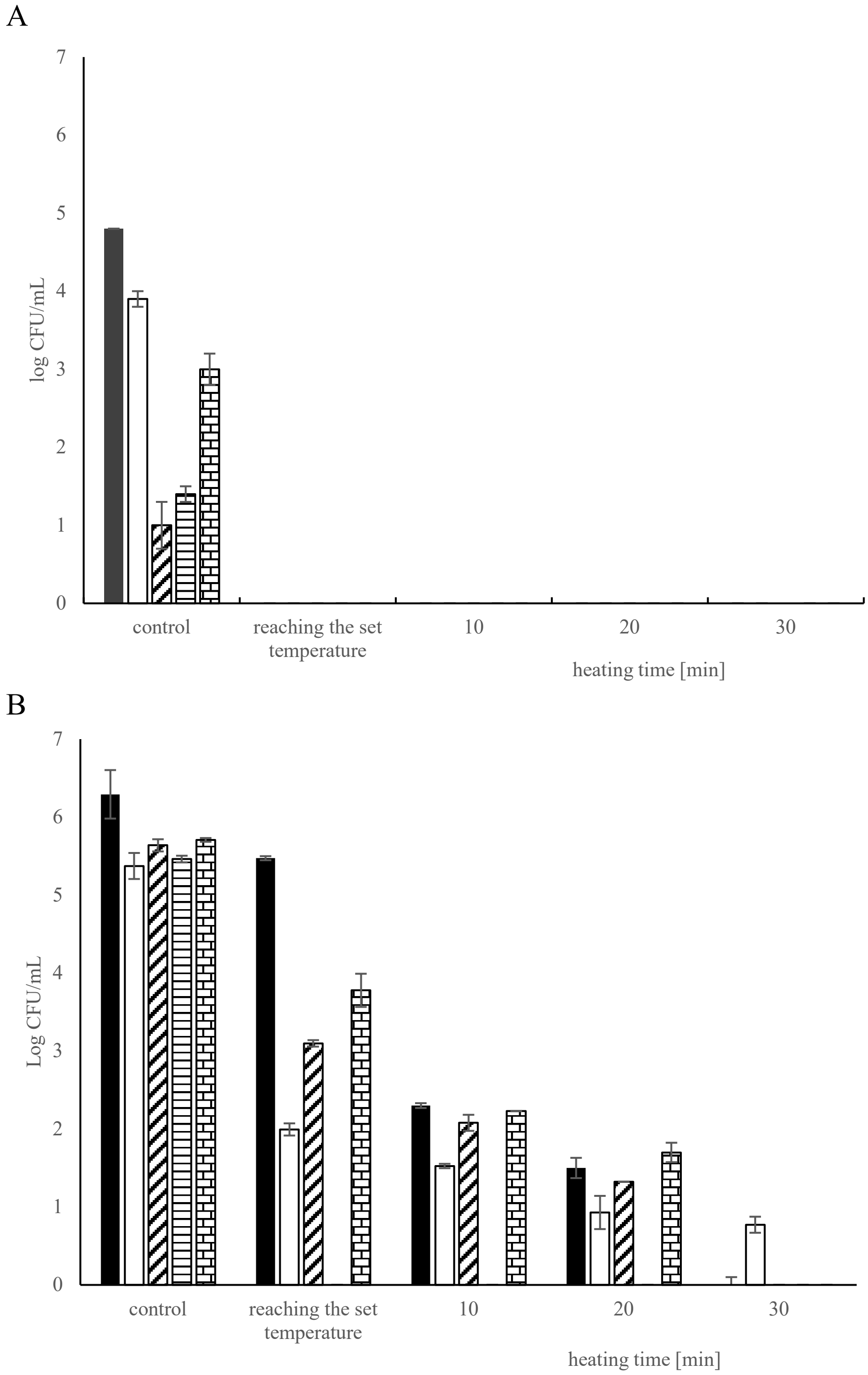

A
log CFU/mL
7
6
5
4
3
2
1
0
control
reaching the set temperature
10
20
30
heating time [min]
B
Log CFU/mL
7
6
5
4
3
2
1
0
control
reaching the set temperature
10
20
30
heating time [min]

Figure 1.

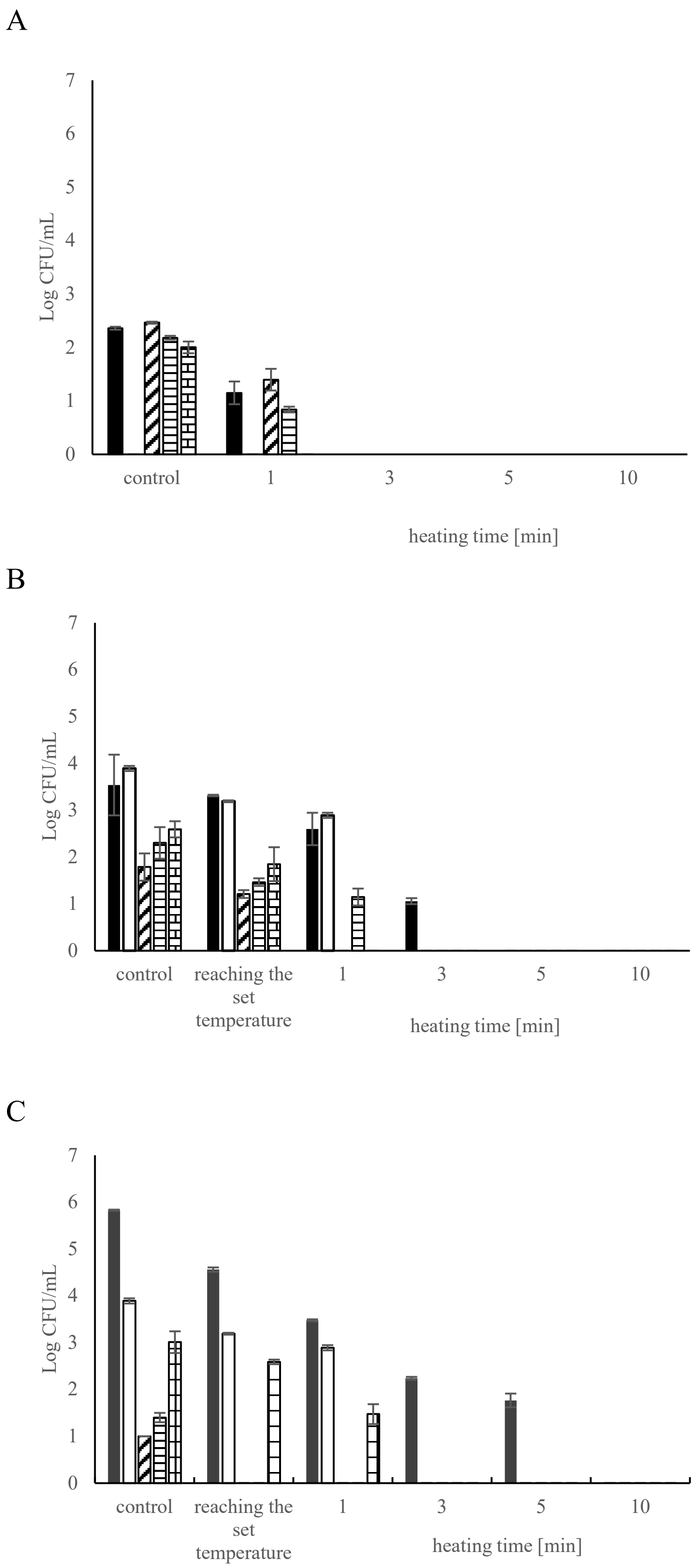
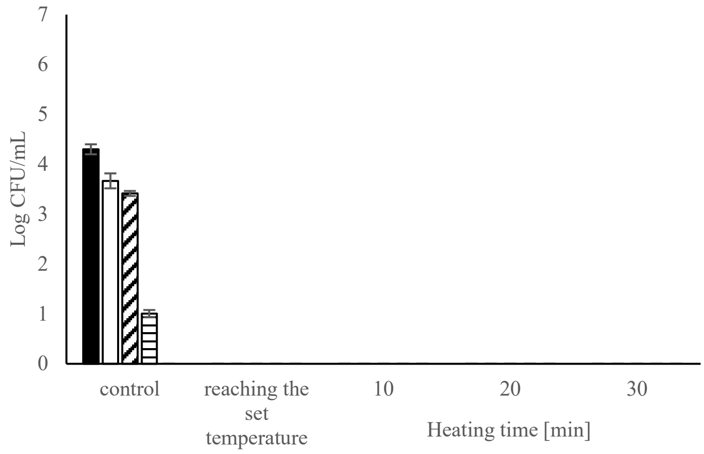

A
7
6
5
4
3
2
1
0
Log CFU/mL
control
1
3
5
10
heating time [min]
B
7
6
5
4
3
2
1
0
Log CFU/mL
control
reaching the set temperature
1
3
5
10
heating time [min]
C
7
6
5
4
3
2
1
0
Log CFU/mL
control
reaching the set temperature
1
3
5
10
heating time [min]

Figure 2.

A


7
6
5
4
3
2
1
0
Log CFU/mL
control
reaching the set temperature
10
20
30
Heating time [min]

B

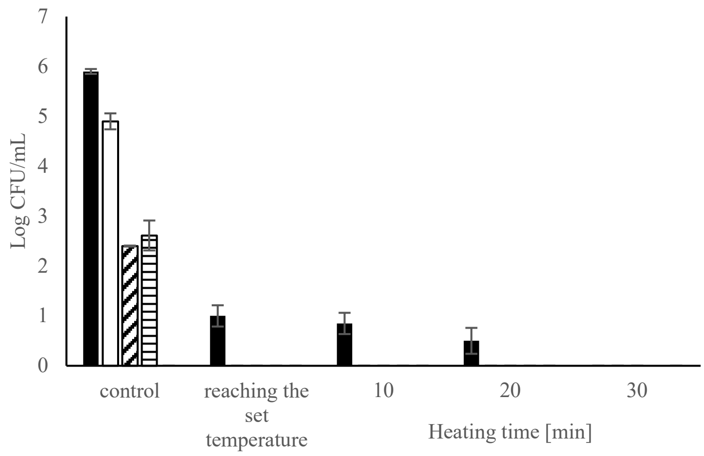
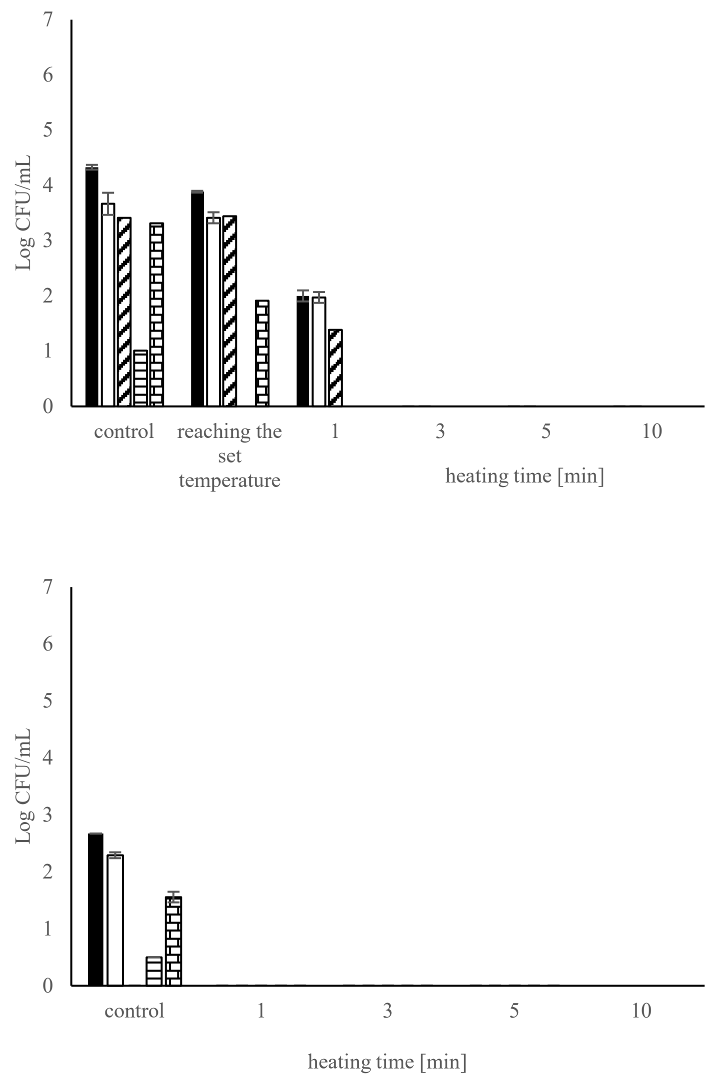

7
6
5
4
3
2
1
0
Log CFU/mL
control
reaching the set temperature
10
20
30
Heating time [min]

Figure 3.

A



7
6
5
4
3
2
1
0
Log CFU/mL
control
reaching the set temperature
1
3
5
10
heating time [min]

B

7
6
5
4
3
2
1
0
Log CFU/mL
control
1
3
5
10
heating time [min]

Figure 4.