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Microwave heat treatment application to pasteurization of human milk

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1 Abstract

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

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15	Industrial relevance: Looking for new methods of donor human milk (HM)
16	preservation is dictated by the necessity of providing microbiological safety and, at
17	the same time, maintain its high nutritional and biological value. The holder
18	pasteurization used in the Human Milk Banks (HMB) (heating at 62°C for 30 min)
19	leads to inactivation of all vegetative forms of microorganisms. Unfortunately, this
20	method causes significant reduction of health benefitting properties of HM. The paper
21	demonstrates the possibility of using the new microwave pasteurizer for preservation
22	of HM, allowing for quick heating of milk to the appropriate temperature and
23	maintaining it in these conditions for a required time. It was shown that the decimal
24	reduction times (D) for strains inoculated to UHT or human milk are several times
25	shorter by using microwave heating than in the commercial pasteurization method.
26	The total inactivation of HM microbiota is obtained after heating at 62.5 and 66°C for
27	5 and 3 min, respectively.
28	

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

31 **1. Introduction**

32 Breastfeeding is unquestionably the best way of feeding newborns and infants. 33 Composition of HM is perfectly adapted in quality and quantity of compounds to the 34 needs of developing children at every stage of their growth. Breast milk provides not 35 only the basic nutrients but also is the source of biological components increasing the 36 child's immunity against bacteria and viruses. A newborn does not yet have a mature 37 immune system, so this first food supplements these deficiencies. 38 There are situations when a baby cannot be directly fed with its mother's milk. 39 This regards to especially premature infants, fed in the initial period only 40 intravenously, and children with extremely low birth weight or health problems. Then 41 it is essential to collect and appropriately preserve the breast milk, so that a baby can 42 be fed with it in the chronological order. This task is carried out at the HMB. HM is 43 not a sterile product and contains microorganisms, which are commonly found in 44 mammary glands and on the skin of the mother. Among them sometimes are 45 pathogenic species such as, for example, S. aureus. Inappropriate handling of milk 46 can also lead to cross-contamination. In many studies the incidental presence of 47 microorganisms in milk, such as Escherichia coli, Klebsiella sp. (Eja, Asikong, Udo, 48 Mboto, & Arikpo, 2006), Acinetobacter (Acinetobacter baumanni, Acinetobacter 49 lwoffii, Acinetobacter haemoliticus), Citrobacter freundii, Serratia liquefaciens and 50 aerobic gram-positive bacilli (Kamianowska, Szczepański, Bebko, Kamianowski, & 51 Milewski, 2008), Listeria monocytogenes (Svabic-Vlahovic, Pantic, Pavicic, & 52 Bryner, 1988) or rickettsia Coxiella burnetii (Kumar, Yadav, & Kakkar, 1981) has 53 been noted. Milk intended for the beneficiaries of HMB should not contain any 54 pathogens and viruses. For this reason, milk in HMB is usually pasteurized at 62.5°C 55 for 30 min (it is so-called holder pasteurization). Heating in these conditions leads to

56	eliminating potentially dangerous microorganisms (viruses and pathogenic vegetative
57	bacteria) from HM. It is carried out in special pasteurizers that make it possible to
58	control the pasteurization process and rapid cooling of the milk to 4°C. The
59	conditions (temperature, time) of holder pasteurization were adopted on the basis of
60	researches conducted at the turn of the XIX/XX century. Based on these studies, in
61	1924, Public Health Reports defined pasteurization as a heating process at
62	temperature not less than 61.1°C by 30 min (Holsinger, Rajkowski, & Stabel, 1997)
63	and was called Low-Temperature-Long-Time (LTLT) pasteurization. This method
64	allows for the effective elimination of most vegetative pathogenic microorganisms,
65	however, leads to a significant decrease of the content of many nutrients and bioactive
66	milk components. Therefore, to guarantee microbiological safety and simultaneously
67	to maintain the high nutritional and biological value of HM, new techniques of
68	preservation are searched for. An alternative method may be the use of High-
69	Temperature-Short-Time (HTST) pasteurization. The possibility of using HTST
70	pasteurization, which would allow for better preservation of biochemical properties of
71	HM than LTLT pasteurization, is rarely tested because of the lack of the necessary
72	equipment for such processing of small amounts of HM (Jensen & Jensen, 1992). The
73	use of HTST rather than LTLT pasteurization is dictated by the principle, which says
74	that the microorganisms are destroyed faster than nutrients during rising of the
75	temperature of pasteurization. This phenomenon is used in food processing, where the
76	goal is to replace the heating at lower lethal temperatures in a longer time with
77	heating at high temperatures and in a shorter time to achieve the same biological
78	effect. This procedure contributes to the protection of nutrients during the heat
79	pasteurization process.

80	The aim of these studies was determination of survival of selected bacterial
81	strains inoculated into cow milk – (UHT, 3.2% of fat) or into sterilized HM (as a
82	model condition), and microbiota of HM, after microwave HTST pasteurization at
83	different temperatures and comparison with traditional HP. A specially designed
84	microwave pasteurizer (Enbio Technology – prototype) was used in these studies.
85	This device differs from common microwave ovens because it allows for
86	pasteurization of small volumes of milk in strictly programmed time (several seconds)
87	at a given temperature. This device has the function of rapid cooling of milk and self-
88	cleaning option. In addition, all experiments were carried out under conditions that
89	can be applied at HMB.
90	2. Material and methods
91	2.1. Materials
92	UHT milk with a fat content of 3.2% from one manufacturer was purchased from a
93	local market. Mature milk was collected from healthy mothers who gave birth on the
94	scheduled date and without complications at the Department of Obstetrics of the
95	Clinical Hospital in Gdańsk. All newborns were in good health (Apgar score of 9 -
96	10) with normal birth weight ($3100 \div 3800$ g). The collected milk was pooled, divided
97	into 50 mL samples and heated at different conditions.
98	All of the experimental procedures were approved by the Local Ethics
99	Committee of the Medical University of Gdansk. The patients gave written consent to
100	participate in the study.
101	2.2. Cultures and growth conditions
102	The following bacterial strains were used: Escherichia coli K-12 PCM2560 (NCTC
103	10538) Pseudomonas geruginosa PCM499 Stanbylococcus gureus PCM 2054
	10550), 1 seudomonus del aginosa 1 CM1+99, Siaphylococcus dal eus 1 CM1 2054
104	(ATCC 25923), Staphylococcus epidermidis PCM 2118, Enterococcus faecalis

- 105 PCM896 and PCM1861, as well as *Enterococcus hirae* PCM2559 and *Enterococcus*
- 106 durans PCM1857 from the Polish Collection of Microorganisms, Ludwik Hirszfeld
- 107 Institute of Immunology and Experimental Therapy of the Polish Academy of
- 108 Sciences, Wrocław, Poland.
- 109 The cultures in stationary phase were prepared by inoculating 100 mL of TSB

110 (tryptic soy broth) with 100 µL of liquid culture (at stationary phase of growth) and

- 111 incubating it at 37 °C for 24 h with shaking.
- 112 **2.3. Preparation of cell suspensions**

113 The cells in the stationary phase of growth were resuspended in UHT milk or in HM,

- 114 previously sterilized at 121°C for 20 min, to give viable counts of about 10⁵ CFU/mL
- 115 of the final concentration. The size of the inoculum has been determined on the basis

116 of the maximum microbial contamination of human milk that is accepted in HMB

117 (Arslanoglu et al., 2010; Malinowska-Pańczyk & Rosiak, 2017).

118 **2.4. Microwave heating**

119 The samples of milk (50 mL) in breastmilk bottles (Medela Ltd.) were placed into the

120 chamber of microwave pasteurizer. The milk was pasteurized using the prototype

121 EnbioJet Microwave Flow Pasteurizer (Enbio Technology Co., Kosakowo, Poland)

122 dedicated to small volume of liquid products (Patent Application no. PL 384854).

- 123 This equipment allows on quick heating of a small volume of milk, and rigorous
- 124 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
- 126 cooled to about 15°C. Triplicate determinations were made for each time and

127 temperature.

128 **2.5. Holder pasteurization**

129 Milk was pasteurized following the procedure of the Human Milk Banking. The 130 samples (50 mL) in breastmilk bottles (Medela Ltd.) were placed in a water bath, 131 heated to 62.5-72°C and keep at this temperature for 0 (immediately after reaching the 132 set temperature), 10, 20 and 30 min. The temperature of milk during heat processing 133 was monitored using a calibrated thermometer placed into control bottle, containing 134 the same volume of non-contaminated milk, and heated in a water bath with all the 135 other samples. After the heat processing, the milk was rapidly cooled in an ice bath 136 and stored prior to determination of viable counts. Untreated samples were used as

137 control.

138 **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.

144 **2.7 Statistical analysis**

145 The data presented in the figures and table are average values of at least three 146 replications with standard deviation. Analysis of variance (one-way procedure) was 147 performed to evaluate differences between treatments using the Statistica 8.0.

148 **3. Results and discussion**

149 **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). 154 The results shown in a Table 3, indicate, that the gram-negative bacteria E. coli 155 and P. aeruginosa and gram-positive bacteria S. aureus and S. epidermidis have been 156 completely inactivated already at the time of reaching the temperature in the range 62.5-157 72°C during HP. Czank, Prime, Hartmann, Simmer, and Hartmann, (2009) showed that 158 holding at 62.5°C caused lowering the number of E. coli, S. epidermidis and S. aureus 159 by 1 log cycle after 5.4 min, 5.6 min and 11.9 min, respectively. These differences 160 between our data and those of Czank et al. (2009) may results from variation in heat-161 resistance of particular strains belonging to the same species as well as from the method 162 of heating of samples (especially from the sample size). In the case of thermoresistant 163 strains Enterococcus sp., the populations decreased as the temperature increased and 164 only after the time of reaching the temperature 72°C all enterococci strains were not detected in 1 mL of sample (Table 3). 165

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:

- 177 $D_T = t / \log_{10} (N_0 / N_t)$
- 178 $k = 2,3026 / D_T$

179 where: N_0 - the initial cell count, N_t - the number of cells after time *t* of heating at 180 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.

187 In the case of thermoresistant enterococci heated by HP, the D_{62.5°C} amounted 188 to 5.67 and 33.2 min for *E. hirae* and *E. durans*, respectively. The number of cells in 189 population of E. faecalis strains decreased only slightly after 30 min treatment in this 190 temperature, therefore, the calculated $D_{62.5^{\circ}C}$ were very high and reached 66.2 min and 191 71.1 min for E. faecalis PCM896 and E. faecalis PCM1861, respectively. The value 192 of the inactivation rates $k_{62.5^{\circ}C}$ was very low for all enterococcus strains. Bacteria 193 belonging to *Enterococcus* sp. are considered, as the most heat-resistant among non-194 spore forming bacteria (Garg & Mital, 1991; Perez, Lorenzo, Garcia, Hernandez, & 195 Ordonez, 1982) and showed only about 0.5 log cycle loss of viability after heating in 196 neutral environment at 60°C by 30 min (Clark, Witter, & Ordal, 1968). At 66°C, the 197 most heat resistant was E. faecalis PCM896. Heating for 12 min is needed to reduce 198 the population of this strain by one log cycle at 66°C. The most sensitive was E. hirae 199 with $D_{66^{\circ}C} = 0.1$ min. Strains *E. faecalis* PCM896, *E. hirae* and *E. durans* were not 200 detected already after reaching of temperature 70 and 72°C and it was impossible to 201 calculate the D-values. Only the strain E. faecalis PCM1861 survived the time to 202 reach temperature of 70°C and D70°C amounted to 0.95 min (Table 3). In turn, Perez et 203 al., (1982) have shown that heating at 64°C results in the inactivation of 90% of the

204 population of E. durans, E. faecium and E. faecalis suspended in cow's milk after 205 13.4, 6.3 and 4.5 min respectively. Similarly to our results, with increasing 206 temperature, the D-values of these strains were shorter. Heating at 72°C led to 207 shortening of the D time to a few seconds. Many factors influence the thermal 208 resistance of enterococci, such as time-temperature combinations and properties of 209 particular strains. It is known that the differences in heat sensitivity can appear not 210 only between species but also among strains within one species and can result from 211 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*.

217 The available literature data regarding the effect of microwave heating on 218 survival of microorganisms are difficult to compare, because the process parameters 219 are often described only by the power unit or the frequency of electromagnetic waves 220 in microwave oven without temperature value. Górecka, Grochowska, Windyga, 221 Ścieżyńska, and Karłowski, (1999) showed that microwave assisted heating (600 W) 222 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 223 224 MHz with a power of 1500 W for 30 min on E. faecalis (initial number of cells was 10^9 225 per mL) reduced the population only by 1 log cycle (Lechowich, Beuchat, Fox, & 226 Webster, 1969). These discrepancies are probably due to different heat resistance of the 227 strains belonging to this species or initial population size. It is known that the lethal 228 effect of temperature is also dependent on level of initial contamination.

229	In the literature there are few data about heat-sensitivity of enterococci in HM
230	(introduced to HM or microbiota). It has been checked whether the same time of
231	heating is sufficient to achieve the total inactivation if the bacteria were suspended in
232	HM instead of UHT milk. In both, UHT milk and HM, complete inactivation of
233	bacteria occurred after the same time of HP (data not shown). In the case of
234	microwave heating enterococci suspended in HM were more resistant than in UHT
235	milk. To achieve total inactivation, longer heating times were needed in some cases
236	(Table 6). As was reviewed by Andreas, Kampmann, and Mehring Le-Doare (2015)
237	and Lis, Orczyk-Pawiłowicz, and Kątnik-Prastowska (2013) the composition of cow
238	milk differ from HM. Especially a content of carbohydrates can affect the protective
239	effect. HM contains 70 g/L of lactose and 7 g/L of oligosaccharides, whereas UHT
240	milk only 48 g/L and 0,1 g/L, respectively.
241	3.2. Effect of heating method on microbiota of breast milk
242	It was demonstrated that the time needed to achieve complete inactivation of

243 microbiota depends on the process temperature and initial microbial contamination of

244 milk. Figures 1, 2, 3 and 4 show survival of natural and cross-contaminated

245 microbiota of HM (TBC, LAB, enterococci, coagulase-positive staphylococci and coli

246 group bacteria). HP at 62.5°C for 30 min did not allow a complete reduction all

247 groups of microorganisms when the initial TBC was above the maximum permissible

248 level (>10⁵ CFU/mL). After this time lactic acid bacteria (LAB) were detected in milk

249 (about 1 log CFU/mL) (Fig. 1B). The use of microwave heating at this temperature

- 250 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
- 252 efficiency of pasteurization at 62.5°C was higher when the initial contamination of

253	milk was lower (Fig. 1A, 2A and B). Bacteria were not detected in 1 mL of milk after
254	reaching 62.5°C during HP or after 3 or 5 min of microwave assisted heating.
255	Increasing the temperature to 66°C allowed to reduce the heating time needed
256	to completely inactivate of the bacteria compared to the effect of 62.5°C. The
257	population of HM microbiota did not survive just after reaching the set temperature
258	(HP) when its number did not exceed 10^5 CFU/mL (TBC) (Fig. 3A). While the initial
259	contamination was greater than 10^5 CFU/mL, the same level of inactivation was
260	possible after 30 min of heating at this temperature (Fig. 3B). Microwave heating at
261	66°C caused total pasteurization effect after 1 or 3 min of heating depending on the
262	initial level of TBC (Fig. 4).
263	Coliform bacteria (similar to <i>E. coli</i> at model conditions) were sensitive to HP
264	conditions and were not detected after reaching of temperature 62.5°C and 66°C (Fig.
265	1 and 3). Microwave pasteurization inactivated the coliforms after 3 min of heating at
266	62.5°C or after reaching of 66°C (Fig. 2 and 4). It was a time shorter than the one for
267	E. coli in model conditions. In the case of coagulase-positive staphylococci when the
268	number of cells was about 10 ³ CFU/mL, HP and microwave heating caused total
269	inactivation after reaching the temperature 62.5 and 66°C (Fig 1A, 2, 3 and 4). A
270	longer time (30 min at 62.5°C) was needed to inactivate all coagulase-positive
271	staphylococci when their number in raw milk was about 10 ⁵ CFU/mL (Fig. 1B).
272	The number of enterococci in most milk samples ranged from 0-10 ³ CFU/mL,
273	except for the sample heated during HP at 62.5°C (Fig. 1B). This group of bacteria
274	was sensitive to heating at 62.5 and 66°C and was not detected usually after reaching
275	the required temperature (Fig 1A and 3A, B). The longest time needed to complete
276	inactivation of enterococci by microwave heating was 3 min (Fig. 2 and 4). In recent
277	years there have been reports that bacteria from Enterococcus genus can be

278 opportunistic pathogens and cause infection especially of hospitalized,

279 immunocompromised patients. Among the species, which caused the disease are

280 mentioned strains of *E. faecalis* and less *E. faecium*. They have the ability of

281 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

283 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

285 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*

287 genus are used as probiotic, which exert positive impact on intestinal tract of humans.

288 Enterococci also produce natural antimicrobial substances with broad-spectrum

inhibiting the growth of pathogenic microorganisms. Both, European and American

290 organizations responsible for food safety did not regulate permissible, acceptable

291 levels of these microorganisms in food products, as well as in breast milk. Due to the

292 lack of virulence among strains found in breast milk, a small number of the

293 population of these bacteria after pasteurization should not raise objections.

4. Conclusions

295 In the last 50 years there have been several reports in the literature regarding 296 the possibility of using the HTST method to preserve HM (Dhar, Fichtali, Skura, 297 Nakai, & Davidson, 1996; Giribaldi et al., 2016; Goldblum et al., 1984; Klotz et al., 298 2017). However, it is difficult to compare the obtained results due to the many 299 differences in the conditions used: temperature, heating time, sample size, challenging 300 test used to determine the effectiveness of pasteurization. Some of the device 301 prototypes used need to be adapted so that they can be routinely used in HMB. In our 302 work we showed that total pasteurization can be achieved in shorter time than by

303	using the holder method. The using of microwave heating allows to inactivate of all				
304	bacterial strains inoculated to human milk and its microbiota. The factor determining				
305	the effectiveness of the pasteurization process is the appropriate microbiological				
306	quality of milk. The contamination of milk with microorganisms above 10^5 CFU/mL				
307	may cause that even 30 min heating at 62.5°C will not effectively eliminate all				
308	bacteria in milk.				
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387 Figure captions

- 388 Figure 1.
- 389 The effect of HP at 62,5°C on viability of microbiota of HM; (■) TBC; (□) LAB; (ℤ)
- enterococci; (目) coli group; (目) coagulase-positive staphylococci;
- 391 A and B mean samples with different initial contamination
- 392 Figure 2.
- 393 The effect of microwave heating at 62,5°C on viability of microbiota of HM () TBC;
- 394 () LAB; () enterococci; () coli group; () coagulase-positive staphylococci;
- A, B and C mean samples with different initial contamination
- 396 Figure 3.
- 397 The survival of microbiota of HM during HP at 66°C; (\blacksquare) TBC; (\square) LAB; (\square)
- enterococci; (目) coli group; (目) coagulase-positive staphylococci;
- 399 A and B mean samples with different initial contamination
- 400 Figure 4.
- 401 The survival of microbiota of HM during microwave heating at 66°C; (■) TBC; (□)
- 402 LAB; (2) enterococci; (=) coli group; (=) coagulase-positive staphylococci;
- 403 A and B mean samples with different initial contamination

Table 1. Media and growth conditions

	Medium	Conditions
Total Bacterial Count (TBC)	Plate count agar	
Lactic Acid Bacteria (LAB)	MRS agar	
Coliform bacteria	Chromocult [®] Coliform agar	30°C for 48 h
Enterococcus sp.	Chromocult [®] Enterococci agar	
Coagulase-positive	Baird-Parker agar	
Staphylococcus sp.		37°C 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]			
6	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}	$3\pm0.5^{b,B}$	$3\pm0.5^{b,C}$

^{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 \pm SD, n = 3)

Strain	Temperature	HP	Microwave heating	
	[°C]]	Log N _r /N ₀	
	62.5	5.9±0.21*	0.3±0.15	
E = 2i K + 12	66.0	5.9±0.13*	3.8±0.17	
<i>E. coll</i> K-12	70.0	5.9±0.15*	3.7±0.30	
	72.0	5.9±0.19*	5.4±0.21*	
	62.5	5.8±0.10*	1.9±0.24	
D gowein agg DCM400	66.0	5.8±0.05*	4.6±0.11	
P. aeruginosa PCM499	70.0	5.8±0.3*	4.3±0.20	
	72.0	5.8±0.25*	4.8±0.13*	
	62.5	5.5±0.18*	3.3±0.25	
$C \rightarrow i d \rightarrow i d \rightarrow D C M 2110$	66.0	5.5±0.14*	$3.4{\pm}0.07$	
S. epiaermiais PCM 2118	70.0	5.5±0.23*	3.5±0.18	
	72.0	5.5±0.15*	5.0±0.13*	
	62.5	5.2±0.15*	0.9±0.18	
C	66.0	5.2±0.20*	3.2±0.02	
S. aureus PCM 2054	70.0	5.2±0.05*	3.5±0.10	
	72.0	5.2±0.04*	4.8±0.05*	
	62.5	< 0.2	< 0.2	
E free also DCM006	66.0	0.5 ± 0.05	-	
E. Jaecalis PCM896	70.0	5.2±0.20*	1.0 ± 0.02	
	72.0	5.2±0.32*	$1.4{\pm}0.05$	
	62.5	< 0.2	< 0.2	
E faccalia DCM1961	66.0	0.7 ± 0.21	3.6±0.30	
E. Jaecan's FCW1801	70.0	4.8±0.20	3.7±0.20	
	72.0	5.3±0.30*	4.9±0.06*	
	62.5	0.62 ± 0.03	<0.2	
E hirac DCM2550	66.0	3.8±0.11	0.5 ± 0.02	
E. nu de l'OWI2559	70.0	5.2 ± 0.40	0.5 ± 0.03	
	72.0	5.4±0.10*	0.6 ± 0.02	
	62.5	<0.2	<0.2	
E durans DCM1957	66.0	0.5 ± 0.16	1.1 ± 0.18	
L. uuruns r CM105/	70.0	5.4±0.24*	1.8 ± 0.06	
	72.0	5.4±0.06*	2.6±0.2	

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

 $\overline{N_r}$ - 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)

	Tommonotumo	Microwave heating	
Species	remperature –	$k [\min^{-1}]$	D _T [min]
	[C]		
	62.5	6.40	0.36
E coli V 12	66	15.35	0.15
$E. COII \mathbf{K}$ -12	70	46.05	0.05
	72	-	0.003
	62.5	7.94	0.29
P. aeruginosa	66	16.44	0.14
PCM499	70	46.05	0.05
	72	-	0.003
	62.5	12.79	0.18
S. epidermidis	66	14.40	0.16
PCM 2118	70	46.05	0.05
	72	-	0.003
	62.5	13.54	0.17
S. aureus PCM	66	23.03	0.10
2054	70	46.05	0.05
	72	-	0.003

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

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

<u>Caracian</u>	Temperature	H	IP	Microwave heating		
Species	[°C]	$k [\min^{-1}]$	D _T [min]	$k [\min^{-1}]$	D _T [min]	
	62.5	0.035	66.2	0.17	13.7	
<i>E. faecalis</i> PCM896	66	0.19	12.1	0.61	3.8	
	70	-	N.d.	1.32	1.75	
	72	-	N.d.	1.50	1.54	
	62.5	0.032	71.1	1.54	1.5	
<i>E. faecalis</i> PCM1861	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	
	62.5	0.406	5.67	0.57	4.07	
E. hirae PCM2559	66	23.02	0.1	0.89	2.6	
	70	-	N.d.	1.35	1.7	
	72	-	N.d.	1.54	1.5	
	62.5	0.07	33.2	0.16	14.7	
<i>E. durans</i> PCM1857	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

	Heating time [min] at temperature									
Strain	62.5°C		66°C		70°C		72°C			
	UHT	HM	UHT	HM	UHT	HM	UHT	HM		
<i>E. coli</i> (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		
<i>S. aureus</i> (4.7±0.12)*	5	5	1	3	1	3	TRT	TRT		
<i>E. faecalis</i> PCM 896 (5.1±0.01)*	_1	_1	10	_1	10	_1	10	10		
<i>E. faecalis</i> PCM1861 (4.7±0.60)*	10	_1	10	_1	3	10	3	3		
<i>E. hirae</i> (4.6±0.08)*	_1	_1	-	_1	5	5	1	5		
<i>E.</i> durans $(4 4+0 01)^*$	_1	_1	10	_1	3	3	5	5		

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

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



Figure 1.







Figure 3.



Figure 4.