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EFFECT OF HEAT TREATMENT ON MICROBIOLOGICAL AND KEEPING QUALITY OF MILK STORED AT $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$

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The heating of milk at 65, 69 and 72°C for 15 s destroyed 85.2, 91.2 and 94.0% of psychrotrophic bacteria, respectively, and reduced total bacteria number by 74.4, 78.4 and 86.3%, respectively. Coliform bacteria were destroyed so effectively, that none was found in 0.1 cm^3 of milk heated at any of the applied temperatures. Thermization at 65°C for 15 s extended the keeping quality of milk to three days at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

INTRODUCTION

It is a long-standing observation that the quality and technological properties of milk stored for several days at low temperatures deteriorate as a result of the development of psychrotrophic bacteria. To counteract this adverse effect of microorganisms, Stadhouders et al. (quoted in [4]) proposed to subject the milk intended for prolonged cold storage in dairy plants to a mild heat treatment, referred to as thermization, which would destroy the psychrotrophic bacteria. Numerous studies [1, 2, 4, 6, 7, 12, 13] demonstrated the positive effect of thermization on milk keeping quality during cold storage. Zall [16], Zall and Chen [17] observed the favourable effect of milk thermization already at dairy farms. Basing on the available research results, the International Dairy Federation recommended that thermization be carried out at $63\text{-}65^{\circ}\text{C}$ for 10-20 s [4].

In Poland thermization is not yet applied, but its use is being considered at large farms and in milk collection centres [15]. It thus seemed advisable to determine the effect of thermization on selected groups of bacteria and keeping quality of milk and to compare its effectiveness with mild pasteurization ($72^{\circ}\text{C}/15\text{ s}$).

MATERIAL AND METHODS

The experiments were performed on herd morning milk obtained from the experimental farm of the Agricultural University (series 1-4) and on bulk milk

obtained from a milk collection centre (series 5). The milk was transported to the laboratory in sterilized cans.

As soon as the milk was delivered, 500 cm³ samples were transferred into sterile Erlenmeyer flasks for analysis, while the remaining milk was thermized at 65 and 69°C for 15 s and pasteurized at 72°C for 15 s in an Alfa-Laval type P-20 HB plate heat exchanger with an attached tube holder. After each heating variant, 500 cm³ portions of the milk were transferred into sterile Erlenmeyer flask, cooled in ice water and stored together with raw milk at 4°C ± 2°C for 3 days. After 0, 24, 48 and 72 h of storage the milk was analyzed for: the total number of aerobic mesophilic bacteria (bouillon agar with milk and glucose; 30°C/3 days), the number of acid forming bacteria (bouillon agar with lactose and China blue according to Mohr; 30°C/3 days), the number of psychrotrophic bacteria (bouillon agar with milk and glucose; 6-7°C/10 days), the coli titre by the fermentation method (medium with lactose, brilliant green and bile; 30°C/2 days). The determinations were carried out according to Polish Standard [10] with the exception of the psychrotrophic bacteria, which were determined according to IDF standard [3]. Also titratable and active acidity as well as methylene blue reduction tests were determined.

In counting the bacteria colonies in the Mohr's medium, bacteria were regarded as acid forming when they formed colonies with a distinct blue colour (sometimes with a dark blue medium around the colonies) and small light blue colonies often with colourless edges.

The results were statistically evaluated using the analysis of variance [14].

RESULTS AND DISCUSSION

The results of five series of experiments are presented in Tables 1-6 and in the Figure.

The initial acidity of the examined milk (Table 1) was 7,20°SH and was within the limits set by Polish Standard [11] for raw milk. The pH ranged within 6,52-6,73. Methylene blue was decolourized in the reductase test after 5,5-7,0 h. According to Polish Standard the results of this test testify about good quality of milk-class A [11]. The microbiological analyses showed (Table 2) that in one milk sample (analysed in series 3) the total number was 75 · 10³ CFU per 1 cm³, while in the remaining samples the figures ranged from 140 · 10³ to 580 · 10³ CFU/cm³. According to microbiological standards of many western countries (in which the total number of bacteria in 1 cm³ of first class milk must not exceed 50 · 10³ CFU), the examined milk was of moderate quality. However, in Poland milk with such total number of bacteria is regarded as of fairly good quality [9]. In most of the samples the number of psychrotrophic bacteria (Table 3) exceeded the limit set in other countries for highest quality milk (10⁴/cm³). The psychrotrophic bacteria constituted 8,0-10,8% of all the bacteria in the milk. The acid forming bacteria in the various samples of the examined milk (Table 4) amounted to 12-75,3% of the total count (Table 2); these figures are omitted in the Tables.

Table 1. Results of acidity determinations and reductase test in raw and heat-treated milk, and changes of these characteristics during storage of milk at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$

Time of storage (h)	Characteristic	Range of values	Raw milk	Milk heated for 15 s at		
				65°C	69°C	72°C
0	total acidity ($^{\circ}\text{SH}$)	min	7.20	7.20	7.20	7.20
		max	7.20	7.20	7.20	7.20
		mean	7.20	7.20	7.20	7.20
24		min	7.40	7.20	7.20	7.20
		max	7.60	7.20	7.20	7.20
		mean	7.48	7.20	7.20	7.20
48		min	7.60	7.40	7.20	7.20
		max	7.80	7.60	7.40	7.20
		mean	7.64	7.48	7.24	7.20
72		min	7.80	7.40	7.20	7.20
		max	8.00	7.60	7.60	7.20
		mean	7.96	7.52	7.28	7.20
0	active acidity (pH)	min	6.52	6.52	6.52	6.52
		max	6.73	6.73	6.73	6.73
		mean	6.66	6.66	6.66	6.66
24		min	6.49	6.52	6.52	6.52
		max	6.71	6.72	6.73	6.73
		mean	6.64	6.66	6.66	6.66
48		min	6.48	6.51	6.52	6.52
		max	6.70	6.71	6.72	6.73
		mean	6.63	6.64	6.65	6.66
72		min	6.46	6.50	6.55	6.52
		max	6.68	6.70	6.71	6.72
		mean	6.60	6.63	6.64	6.65
0	methylene blue reductase test (h)	min	5.5	> 7	> 7	> 7
		max	7.0	> 7	> 7	> 7
		mean	6.5	> 7	> 7	> 7
24		min	5.0	> 7	> 7	> 7
		max	7.0	> 7	> 7	> 7
		mean	6.2	> 7	> 7	> 7
48		min	4.5	> 7	> 7	> 7
		max	6.0	> 7	> 7	> 7
		mean	5.6	> 7	> 7	> 7
72		min	4.0	> 7	> 7	> 7
		max	5.0	> 7	> 7	> 7
		mean	4.8	> 7	> 7	> 7

Table 3. Number of psychrotrophic bacteria ($\times 10^3/\text{cm}^3$) in raw and heat-treated milk, and changes of this number during storage of milk at $4^\circ\text{C} \pm 2^\circ\text{C}$

Series	Time of storage (h)	Raw milk		Milk heated for 15 s at					
				65°C		69°C		72°C	
		number	%	number	%	number	%	number	%
1	0	15.10	10.80	0.59	1.2	0.45	1.1	0.35	2.2
2		15.50	8.30	0.71	4.0	0.33	4.1	0.22	3.4
3		7.20	9.70	1.53	26.8	1.20	27.9	0.88	29.2
4		39.50	8.00	4.30	10.1	0.51	3.1	0.26	2.3
5		52.00	9.00	12.00	4.5	8.90	3.6	6.00	3.6
1	24	63.50	14.8	0.94	1.9	0.88	2.2	0.73	2.2
2		56.50	19.8	1.79	4.1	0.67	4.2	0.43	3.3
3		18.00	15.8	2.08	27.2	1.57	28.3	1.20	30.0
4		129.00	19.7	6.10	15.1	0.72	4.0	0.27	1.9
5		135.00	15.2	16.50	5.5	11.50	4.2	7.10	3.7
1	48	109.00	18.9	1.68	2.4	1.30	2.3	0.82	2.3
2		114.00	25.1	5.30	8.6	2.26	9.2	0.56	3.5
3		165.00	44.0	3.30	28.7	2.05	28.9	1.20	31.3
4		523.00	29.1	7.10	15.4	1.36	5.0	0.35	2.8
5		535.00	23.2	23.00	6.4	21.00	5.7	12.00	4.3
1	72	205.00	25.6	2.34	2.5	1.98	2.5	1.20	2.5
2		315.00	30.6	8.85	11.1	3.60	11.8	0.94	4.4
3		350.00	69.5	4.35	31.1	3.00	31.6	2.40	36.4
4		1550.00	43.7	14.60	15.4	4.60	15.9	1.05	5.1
5		1400.00	41.8	22.00	6.2	18.50	5.3	10.20	4.2

*) per cent of the total number of bacteria (cf. Table 2)

The coli titre was 0,01 in all the milk samples (data not shown in the tables).

Data concerning the numbers of the examined groups of bacteria in heat treated milk are given in Tables 2-4 and the percentages of bacteria destroyed during heating are listed in Table 5. As can be seen from Table 2, the total number of microorganisms in the individual milk samples varied fairly significantly depending mainly on the quality of the raw milk (as for example in milk samples 3 and 5). In four samples of heat-treated milk (series 1-4), the total number of bacteria was below $50 \cdot 10^3 \text{ CFU}/\text{cm}^3$, and only in one sample (from milk collection centre—series 5) the number ranged from $165 \cdot 10^3$ to $265 \cdot 10^3 \text{ CFU}/\text{cm}^3$ depending on heating temperature. Noteworthy is the varying degree of destruction of bacteria during heat treatment of the individual milk samples (Table 5), depending apparently on the varying share of heat-resistant bacteria in the microflora of the investigated milk.

The F_{emp} coefficient calculated for total number of bacteria in heated and raw milk was 159.8, with the $F_{tab.}$ value was 4.13 ($\alpha = 0.01$) [14]. This means that each of the applied temperatures had a statistically significant effect on the mean number of bacteria. On the other hand, a comparison (Figure) of differences

Table 4. Number of acid forming bacteria ($\times 10^3/\text{cm}^3$) in raw and heat-treated milk, and changes of this number during storage of milk at $4^\circ\text{C} \pm 2^\circ\text{C}$

Series	Time of storage (h)	Raw milk	Milk heated for 15 s at		
			65°C	69°C	72°C
1	0	92.0	30.0	29.0	10.5
2		149.0	14.2	4.3	2.4
3		24.0	3.7	1.4	1.2
4		60.0	4.2	3.7	2.8
5		260.0	125.0	95.0	96.5
1	24	35.0	32.5	27.0	10.5
2		26.2	14.3	13.2	2.6
3		4.4	3.5	3.2	2.7
4		16.0	6.1	5.1	2.2
5		174.0	173.0	162.0	102.0
1	48	33.5	29.0	29.0	11.0
2		38.5	35.0	7.9	2.5
3		4.2	2.9	2.1	2.6
4		13.2	11.8	4.5	3.7
5		204.5	194.0	129.0	100.0
1	72	95.0	27.5	27.0	11.5
2		91.5	40.0	7.1	2.2
3		135.0	3.2	2.6	2.9
4		1.905.0	5.8	2.9	2.6
5		450.0	194.0	129.0	100.0

Table 5. Percentages of bacteria destroyed during the heating of milk at various temperatures for 15 s

Series	Total number			Psychrotrophic bacteria			Acid forming bacteria		
	65°C	69°C	72°C	65°C	69°C	72°C	65°C	69°C	72°C
1	65.4	71.1	88.1	96.6	97.1	97.7	67.4	69.5	88.6
2	90.4	95.7	96.5	95.4	97.9	98.6	88.7	96.7	98.1
3	92.4	94.7	96.0	79.0	83.5	87.9	84.6	94.2	95.0
4	91.3	96.7	97.7	89.1	98.7	99.4	93.0	93.8	95.4
5	54.4	56.9	72.4	76.9	82.9	88.5	52.0	64.5	62.9

between mean numbers of bacteria in milk samples heated at various temperatures with the least significant difference shows that there are no significant differences between the different variants of heat processing as regards destruction of bacteria. An interesting observation is that $65^\circ\text{C}/15\text{ s}$, the upper temperature limit envisaged by IDF for milk thermization [4], had similar bactericidal effect as mild pasteurization ($72^\circ\text{C}/15\text{ s}$). As shown in Table 5, the $65^\circ\text{C}/15\text{ s}$ treatment destroyed 54.3-92.4% of the total number of microorganisms; the figure for the 69°C treatment was 56.9-96.7%, and for the $72^\circ\text{C}/15\text{ s}$

treatment — 72.4-96.5 %. In three milk samples (series 2, 3, and 4) heating at all the temperatures destroyed over 90 % of the total number of microorganisms. In studies of other researchers, the effectiveness of destruction of bacteria during milk thermization was slightly higher. For example Kimenai (quoted in [4]) heated milk samples with various initial microorganisms numbers ($50 \cdot 10^3/\text{cm}^3$, $80 \cdot 10^3/\text{cm}^3$ and $1500 \cdot 10^3/\text{cm}^3$) at 60, 62, and 64°C for 12.5, 15, and 20 s, and in this way destroyed 90-99.1 % of the microorganisms, with the greatest proportions of microorganisms being destroyed in milk with the highest total number of bacteria.

In our experiments the psychrotrophic bacteria turned out to be highly sensitive to heating, their number being reduced to a greater degree than the total number (Tables 3 and 5). Compared with the psychrotrophs number in raw milk, each variant of heat treatment led to a statistically significant drop of this number. The calculated F_{emp} coefficient was 99.1, while $F_{tab.}$ was 4.13. There were no statistically significant differences between the mean numbers of psychrotrophic bacteria in milk samples heated at various temperatures (Figure). The numbers of psychrotrophic bacteria which survived in the milk, and which may be considered as heat-resistant psychrotrophs, were of the order of several thousand CFU/cm³ or even several hundred CFU/cm³ (Table 3). Their proportion in the total number of microorganisms varied somewhat in the individual milk samples, but was usually lower in milk heated at higher temperatures. The presence of heat-resistant psychrotrophs was also reported by other authors [2, 4]. The coliform bacteria were so effectively eliminated during all variants of heat treatment that they were absent in 0.1 cm³ milk samples directly after heating.

The acid forming bacteria were fairly resistant to heating (Tables 4 and 5). Dominant among the colonies were small light blue colonies of weakly acidifying microbacteria and micrococci which, as is well known, are heat-resistant. When viewed under a microscope, the colonies were found to contain short not motile rods and cocci. Transferred to litmus milk the selected colonies did not coagulate the milk, but only changed the colour of the litmus after 24 h of incubation at 30°C . Given the fact that the blue colonies were formed not only by typical acid forming bacteria (i.e. lactic streptococci) but also by other bacteria, the precise determination of the number of the former was not easy. This determination was regarded as approximate and no statistical evaluations were done.

The performed experiments demonstrate that thermization as well as heating at higher temperatures greatly reduced the microbiological contamination of milk, this being indicated both by the microbiological determinations and by the reductase test.

The assumed criterion of thermization effectiveness is the difference between the total number of bacteria in the milk directly after treatment and the number of bacteria after three days of storage at temperatures not exceeding 8°C in conditions preventing reinfection [4]. An important indicator are changes in bacterial number in raw and heat-treated milk stored in identical conditions.

Looking at our experiments from this viewpoint, we may say that thermization at 65°C for 15 s extended the keeping quality of milk to 3 days at 4°C ± 2°C. Raw milk stored at 4°C maintained good quality only during 24 h. On further storage statistically significant increase of total count was observed. The coli titre however, remained on the initial level.

Table 6. Mean increment (in per cent) of bacteria in raw and heat-treated milk stored at 4°C ± 2°C

Time of storage (h)	Raw milk	Milk heated for 15 s at		
		65°C	69°C	72°C
		total number		
24	62.5	16.6	11.1	25.0
48	273.3	45.8	50.8	71.9
72	523.6	58.8	53.9	67.8
		number of psychrotrophic bacteria		
24	310.8	43.3	34.7	26.2
48	930.3	111.1	143.7	98.4
72	2.837.8	172.6	151.9	105.0

LSD least significant difference

The bacteria most dynamically developing during cold storage were psychrotrophs (Tables 3 and 6, Figure). Already after 24 h the number of psychrotrophs increased by about 3 times, and after 72 h the increase was 28-fold. In consequence the share of these bacteria in the total number increased to 26-70% after 3 days. With such a high number of psychrotrophs in the milk, one may expect significant changes of some of its components and the production of heat-resistant lipases and proteases [7].

The acid forming bacteria multiplied relatively slowly during milk storage at 4°C, which is understandable taking into account their mesophilic character (Table 5).

The total acidity of raw milk reached after 24 h 7.5°SH, i.e. the maximum level allowed by Polish Standard for raw milk [11]. Compared with this, the active acidity of milk remained pretty stable and after 72 h of storage it was almost the same as in fresh milk (Table 1). Comparing the increase of total acidity with the number of bacteria in the milk (especially of acidifying bacteria) it is obvious that this increase cannot be due entirely to the action of bacteria. The increased acidity may be partly explained by lipolytic changes caused by native milk lipase.

As regards the growth of bacteria in the heat-treated milk, the time of storage had no significant effect on either the increase of total number ($F_{emp.} = 1.2$, $F_{tab.} = 2.04$) or the number of psychrotrophic bacteria ($F_{emp.} = 1.2$, $F_{tab.} = 2.72$). This is clearly apparent in the Figure. The increase of the total number of bacteria was on average about 9 times lower than in raw milk, and the number of psychrotrophs—about 16 times lower. The total number of bacteria not

exceeding $360 \cdot 10^3$ CFU/cm³ in milk heated at 65°C for 15 s after 72 h of storage (and still less psychrotrophic and acidifying bacteria) was not high enough to adversely affect its technological properties. It may also be noted that coliform bacteria were absent in 0.1 cm³ heat-treated milk samples after 3 days of storage.

The acidity of the investigated milk increased only slightly in the milk heated at 65°C and 69°C for 15 s and stored for 72 h. There were no acidity changes in pasteurized milk.

The result obtained in this work confirmed the opinion of many authors [1, 2, 4, 6, 7, 12, 13] about the favourable effect of thermization on microbiological quality and keeping quality of milk. In Fonden's [57] study thermization at 65°C for 15 s preserved milk quality for 48 h of storage at 5°C. At the end of this period, thermized milk contained not more than $4 \cdot 10^3$ CFU of bacteria while in raw milk the number was $1 \cdot 10^6$ - $22 \cdot 10^6$ CFU/cm³. By thermizing milk at the same temperature Coghill et al. [2], Gilmour et al. [6], Griffiths et al. [7] and Senyk et al. [12] extended its keeping quality to 3-4 days during storage at both 4°C and about 7°C [12]. The heat-resistant bacteria (including psychrotrophs) that remain in the milk show a slow growth rate in such conditions and do not deteriorate the milk's quality [4], a fact also observed in this research. Considering the fact that the most harmful bacteria such as psychrotrophs are destroyed already after 15 s of heating at 65°C, it does not seem necessary to use higher temperatures. Bjorgum et al. [1], however, express the opinion that milk of low initial quality should be subjected to more severe heat treatment (69-72°C). Van den Berg [4] reports, for example that in some countries milk is thermized at 68°C/15-20 s.

CONCLUSION

1. The applied variants of heat treatment of milk (65, 69, and 72°C for 15 s) did not show statistically significant differences as regards the reduction of total number and psychrotrophic bacteria.

2. In milk samples heated at the above temperatures, the number of psychrotrophic bacteria was reduced by an average of 85.2, 91.2 and 94.0%, respectively, whereas in the case of total bacteria number the respective figures were 74.4, 78.4 and 86.3%.

The lethal effect of the above variants of heat treatment against coliform bacteria was also very high.

3. Thermization at 65°C for 15 s of milk of moderate microbiological quality extended its keeping quality to at least 3 days at 4°C ± 2°C.

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WPLYW OBRÓBKI TERMICZNEJ MLEKA NA JEGO JAKOŚĆ MIKROBIOLOGICZNĄ I TRWAŁOŚĆ PODCZAS PRZECHOWYWANIA W TEMP. $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$

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Streszczenie

Mleko zbiorcze z gospodarstwa doświadczalnego SGGW-AR oraz z punktu skupu poddawano obróbce termicznej w temp. 65°C , 69°C i 72°C przez 15 s w laboratoryjnym aparacie płytowym. Część mleka surowego jak i poddanego obróbce termicznej przechowywano następnie w temp. $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ w ciągu 3 dni. Po czasie 0, 24, 48 i 72 h mleko poddawano analizie, głównie mikrobiologicznej. Stwierdzono, że stosowane warianty obróbki termicznej mleka nie różniły się pomiędzy sobą statystycznie istotnie pod względem zniszczenia bakterii ogółem i psychrotrofowych. W mleku poddanym stosowanym wariantom obróbki cieplnej stopień zanieczyszczenia bakterii ogółem wynosił odpowiednio 74,4%, 78,4% i 86,3%, zaś bakterii psychrotrofowych 85,2%, 91,2% i 94,0%. Bakterie z grupy coli uległy zniszczeniu w takim stopniu, że były nieobecne w $0,1\text{ cm}^3$ ogrzewanego w różnej temperaturze i przechowywanego mleka. Dynamika przyrostu ogólnej liczby bakterii była w czasie przechowywania mleka ogrzewanego 9-krotnie niższa, a psychrotrofów ok. 16-krotnie niższa niż w mleku surowym. Termizacja mleka (o średniej jakości mikrobiologicznej) w temp. $65^{\circ}\text{C}/15\text{ s}$ pozwoliła na przedłużenie jego trwałości na okres co najmniej 3 dni w temp. $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, dzięki zniszczeniu znacznej części zawartych w nim bakterii.