

Influence of Different Time/Temperature Combinations of Milk Pasteurization on its Bacterial Content

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SUMMARY

The effect of different time/temperature combinations of milk pasteurization on its bacterial content has been studied under laboratory conditions. The results obtained indicated that the bacterial content of the milk was markedly decreased after pasteurization. The general trends in the rate of destruction of bacteria during pasteurization differs according to the type and degree of the initial infection. Milk pasteurization have caused a change in the proportions of bacterial species towards a predominantly heat resistant microflora. The main types of bacteria surviving various time/temperature combinations were, *Bacillus*, *Microbacterium* and *Micrococcus*.

INTRODUCTION

A greater understanding and appreciation of the microbiological quality of raw and pasteurized milk has developed in the last decade, particularly in relation to product quality. The widespread use of pasteurized milk has brought new bacteriological problems. The milk pasteurization temperatures have caused a change in the proportions of bacterial species towards a predominantly heat-resistant microflora in milk, thus causing undesirable chemical changes in milk and reducing its quality. This has resulted in an increase interest in the problems of quantitative and qualitative composition of heat resistant bacteria in pasteurized milk. Gavel (1960), Desai and Natarajan (1980), Desai et al (1980) and Blankenagel (1982) have shown that there is a close relationship between the number of heat-resistant bacteria in the raw milk and the degree of the destruction of the microflora during the pasteurization of milk.

Therefore, this study was conducted to investigate the influence of different time/temperature combinations of milk pasteuriza-

tion on its bacterial content. Special attention was drawn to heat-resistant bacteria.

MATERIALS AND METHODS

Fresh raw milk collected from 54 cows of the Friesian Breed, obtained from morning milking at the Agricultural Experimental Station. The milk was supplied 3 to 4 hours after milking in the amount of 30 to 40 L in cans previously sterilized in autoclave. The milk was pasteurized at the temperatures of 72°, 76° and 84°C for 15 sec. under laboratory conditions. The experiments had been carried out five times. The total number of microorganisms was determined in the raw and pasteurized milk by using the International Standard FIL/IDF 3/1958. Count of acid forming bacteria was carried out according to Chamba et al. (1981). Count of caseolytic bacteria was performed as previously mentioned by Bernard (1948). Count of Coliform bacteria was determined by the International Standard FIL/IDF 73/1974. The number of heat-resistant bacteria was determined by the plate method after heating milk at 63°-65°C for 30 min. The time of incubation was 5 days at 30°C.

After counting the colonies of heat-resistant bacteria on the plates, one plate with a number of colonies ranging from 30 to 40 was selected from each of raw and pasteurized milk, and 1/3 of this number was inoculated in litmus milk, incubation of litmus milk was at 30°C for 7 days, their action was observed and next the bacteria were submitted to further studies which will be discussed in detail in the present paper.

RESULTS AND DISCUSSION

The results of the five series of tests are listed in Tables 1, 2 and 3. The milk pasteurization temperatures have been selected in accordance with the current practice of the dairy industry, in the cheese-making practice

milk is heated at 72° - 76°C for 15 - 20 sec., whereas in the production of milk for direct consumption, it is heated to a temperature exceeding 80°C. Because of the low bactericidal efficiency of the temperature of 72°C, it was suggested to heat the milk at 74°C or

even 76°C, (Kandler, 1960). It was found useful to compare the action of pasteurization at 72° and 76°C respectively especially from the point of view of heat-resistant bacterial destruction.

Table 1 : Results of the microbiological analysis of raw and pasteurized milk.

Type of Determination	No. of experiment	Raw milk	Pasteurized milk		
			72°C/15 Sec.	76°C/15 Sec.	84°C/15 Sec.
Total number of bacteria in 1 ml. x 10 ³	1	2100	21.0	10.5	4.8
	2	1270	41.0	14.0	2.0
	3	1260	36.0	12.0	1.0
	4	17500	890.0	95.0	0.9
	5	21000	770.0	36.0	1.3
Number of acid forming bacteria in 1 ml x 10 ³	1	2000	1.1	0.7	0.0
	2	1250	1.5	0.09	0.0
	3	900	1.1	0.08	0.0
	4	14900	30.9	0.8	0.0
	5	20900	64.0	1.1	0.0
Number of Caseolytic bacteria in 1 ml x 10 ³	1	350.0	16.1	6.1	0.05
	2	13.0	1.8	1.05	0.009
	3	270.0	15.9	5.6	0.0
	4	14.5	1.5	0.16	0.1
	5	15.0	3.5	3.3	0.0
Number of heat resistant bacteria in 1 ml. x 10 ³	1	27.0	22.0	16.0	9.0
	2	7.6	4.6	2.4	0.8
	3	34.4	28.6	10.2	0.9
	4	1.7	1.3	1.0	0.4
	5	2.5	2.2	1.7	1.2
Coli iter	1	10 ⁻⁵	0.0	0.0	0.0
	2	10 ⁻⁴	0.0	0.0	0.0
	3	10 ⁻⁵	0.0	0.0	0.0
	4	10 ⁻⁵	0.0	0.0	0.0
	5	10 ⁻⁶	0.0	0.0	0.0

As shown by the data listed in Table 1, the milk used for the present study was characterized by a generally low microbiological quality, because of the high total number of bacteria and of bacteria belonging to the coli group in particular.

The results obtained are in good agreement with those obtained by Desai and Natarajan (1980), Desai et al (1980), Collins (1981) and Blankenagel (1982), and highlight the importance of improving the bacteriological quality of raw milk because of its considerable effect on the quality of milk pasteurized at lower temperatures, especially at 72°C. The strongly contaminated milk (samples 4 and 5) contained a comparatively high number of bacteria (several hundred thous./ml) which survived in this temperature in spite of the insignificant contribution of heat-resistant bacteria (Table 2). The overall bactericidal effectiveness of this pasteurization was low even in milk containing comparatively low total number of bacteria (Table 3). An increase of the heating temperature from 72° to 76°C caused an average 10-fold decrease of the total number of bacteria, the effectiveness of treatment was higher in milk containing a large initial number of bacteria (10 - 20 fold decrease of total number), than in milk with lesser initial contamination with microflora (2 - 3 fold decrease).

The results obtained indicated that pasteurization of milk at 76°C/15 sec. enabled to obtain milk containing less than 10⁵ bacteria/ml. and the effectiveness of pasteurization was slightly over 99 %. This results confirmed the earlier results of Kandler (1960), who pointed out to the need of increasing the pasteurization temperature of milk to at least 74°C in order to achieve a product containing not more than 10⁵ cell/ml. Also the results obtained allow to draw the conclusion that the temperature of 76°C/15 sec. is too mild for pasteurization of heavily contaminated milk.

In the same time the temperature of 84°C/15 sec. had a strong bactericidal effect (99.99 %). The number of bacteria present in milk pasteurized at this temperature did not depend on the quality of the raw milk

The results listed in Table 3, show that the efficiency of the temperature of 72° and 76° is slightly higher on acid forming bacteria as compared to the total number. Acid forming bacteria were completely destroyed only when milk was heated at 84°C/15 sec.

The level of contamination of the milk studied with caseolytic and heat-resistant bacteria showed no relationship to the total number of microflora. On the other hand, there was a clear inter-relation between the number of the groups discussed in raw and pasteurized milk, what was recorded also in other experiments especially in relation to heat-resistant bacteria (Gavel, 1960). The observed low effectiveness of pasteurization with regard to both these groups of bacteria (Table 3) resulted in a change of the relations between the various groups of the microflora in the heated milk (Table 2). The significant decrease of the share of or destruction of acid forming bacteria at 84°C was accompanied by a considerable increase of the share of caseolytic bacteria, 7.8 % in raw milk and 25.1 %, 24.8 % and 2.5 % in milk pasteurized at 72°, 76° and 84°C., as well as heat-resistant bacteria, 0.09 % in raw milk and 38.1 %, 41.9 % and 73.1 % in milk pasteurized at 72°, 76° and 84°C respectively, a fact confirmed by other investigators, Oldenburg, (1960) and Schonberg, (1960).

Pasteurization of milk at 84°C/15 sec. caused a very substantial reduction of the participation of caseolytic bacteria in the microflora of the milk, 7.8 % in raw milk and 2.5 % in milk pasteurized at 84°C/15 sec., although it did not eliminate entirely these bacteria from the milk.

Heat-resistant bacteria constitute the most interesting group in the process of the heat treatment of milk. With the raising of the heating temperature, heat-resistant bacteria accounted for an ever higher percentage of the microflora, from 0.09 % in raw milk to 73.1 % in milk pasteurized at 84°C/15 sec. The effectiveness of the temperatures used in the present work was the lowest for this group, from 22.1 % in milk pasteurized at 72°C to 77.1 % in milk pasteurized at 84°C. For this

reason, heat-resistant bacteria was a dominating group in pasteurized milk microflora.

The observed results in the first experiment, higher number of heat-resistant bacteria as compared with the total number of the microflora determined in the same raw milk, can be explained by the breaking up of the natural agglomerations of the cells during

the additional heating of milk at 63° - 65°C/30 min. required for the determination of this group of bacteria. In this case heat-resistant bacteria accounted for 100 % of the microflora of pasteurized milk. This figure has been listed in Table 2 in spite of the fact that calculation pointed to a much higher proportion of heat-resistant bacteria when compared to the total number of bacteria.

Table 2: Percentage of the determined groups of bacteria in the total number of bacteria in raw and pasteurized milk.

Type of Determination	No. of experiment	Raw milk	Pasteurized milk		
			72°C/15 Sec.	76°C/15 Sec.	84°C/15 Sec.
Acid forming bacteria	1	95.2	5.2	6.6	0.0
	2	98.4	3.7	0.6	0.0
	3	71.4	3.1	0.7	0.0
	4	85.1	3.5	0.8	0.0
	5	99.5	8.3	0.3	0.0
Average		89.9	4.2	1.8	0.0
Caseolytic bacteria	1	16.7	76.7	58.1	1.0
	2	1.0	4.3	7.5	6.4
	3	21.4	44.2	46.7	0.0
	4	0.1	0.2	0.2	11.1
	5	0.1	0.4	9.2	0.0
Average		7.8	25.1	24.8	2.5
Heat-resistant bacteria	1	1.3	100.0	100.0	100.0
	2	0.6	11.2	17.1	40.0
	3	2.7	79.1	86.7	90.0
	4	0.01	0.2	1.1	43.2
	5	0.01	0.3	4.7	92.3
Average		0.09	38.1	41.9	73.1

Characteristics of Heat-Resistant Bacteria:

Tests conducted on isolated strains were aimed at the identification and assessment of their share in heat-resistant microflora. Rod-shaped bacteria and cocci were identified on the basis of preliminary microscopic examination stained by Gram's method. The both groups were tested separately.

The tests carried with cocci include determination of their ability of produce catalase and survival at the temperature of 63° - 65°C for 30 min. In the case of rod-shaped bacteria, their ability to produce catalase and survival at 63° - 65°C for 30 min., and 85°C for 15 min. was determined.

A total of 187 strains were isolated from raw and pasteurized milk and examined. Observations under the microscope showed that 166 strains (88.77 %) belonged to rod-shaped bacteria and 21 strains (11.23 %) to cocci. The presence of chains was observed in 6 strains of cocci, the remaining 15 strains

occurred in the form of diplococci, tetrads and of irregular agglomerations resembling grapes. All the strains of cocci were gram positive, produced catalase and survived the temperatures of 63° - 65°C for 30 min. On this basis, 15 strains were classified as belonging family Micrococaceae, genus Micrococcus, ac-

Table 3: Bactericidal effectiveness of milk pasteurization temperatures used.

Type of determination		Pasteurized milk		
		72°C/15 Sec.	76°C/15 Sec.	84°C/15 Sec.
Total number of bacteria	minimum	94.90	98.90	99.30
	maximum	99.00	99.80	99.99
	Average	96.80	99.30	99.91
Acid forming bacteria	minimum	99.40	99.60	100
	maximum	99.90	99.99	100
	Average	99.70	99.99	100
Caseolytic bacteria	minimum	76.70	78.00	99.30
	maximum	95.40	98.30	100
	Average	88.40	92.90	99.80
Heat - resistant bacteria	minimum	12.00	29.60	52.00
	maximum	39.50	68.40	97.4
	Average	22.10	42.4	77.10

extreme and mean values from 5 series of experiments.

Table 4: Composition of heat-resistant bacteria in raw and pasteurized milk.

Tested milk		Total number of isolated bacteria	Microb-		Miscell-aneous
			Bacillus	acterium	
Raw	Number	61	34	16	3
	%	100	55.7	26.2	4.9
72°C/15 S.	Number	51	30	10	8
	%	100	58.8	19.6	15.7
76°C/15 S.	Number	48	31	12	3
	%	100	64.6	25.0	6.3
84°C/15 S.	Number	27	18	8	1
	%	100	66.7	29.6	3.7
Total	Number	187	113	46	15
	%	100	60.4	24.6	8

according to Bergey's (1974). Genus *Staphylococcus* belonging to the same family was excluded in view of its known thermal sensitivity. The remaining 6 strains of *Streptococcus* whose affiliation to genus *Micrococcus* had been excluded.

Among the rod-shaped bacteria, there were observed 113 strains (60.4 %) of spore-forming bacteria representative of the *Bacillus* genus, on the basis of their survival at 85°C for 15 min. and aerobic character of growth.

From the remaining 53 strains (28.3 %), 46 strains were belonged to the typical heat-resistant, asporogenous rod-shaped bacteria among which, according to earlier studies (Gavel, 1960 and Kandler, 1960), *Microbacterium* is the dominating genus. The remaining 7 strains of heat-resistant rods were made unidentified.

The results of the identification of the isolated bacteria in reference to the individual samples of tested milk are presented in Table 4. The results obtained are in good agreement with those reported by Gavel (1960), Kandler (1960) and Collins (1981). The data listed in Table 4 show that the same bacteria as observed in raw milk, are present in milk pasteurized at 72° and 76°C. for 15 sec. but in a different quantitative arrangement. In all cases, however, the main part of heat-resistant microflora in milk was constituted by bacteria belonging to genus *Bacillus* and *Microbacterium*. The third place was occupied by bacteria of the genus *Micrococcus* which were gradually destroyed in the higher temperatures. In milk heated at 84°C for 15 min. they were isolated only once, and as many as 96 % of the microflora was constituted by genus *Bacillus* and *Microbacterium*. In this respect, the results obtained confirm those of Kandler (1960), Abo Elnaga (1966) and Collins (1981), especially concerning *Microbacterium*.

CONCLUSIONS :

The results obtained have showed that :

1 — The effectiveness of milk pasteurization at the temperatures of 72°, 76° and 84°C

for 15 sec. amounted in the average of 96.8, 99.3 and 99.91 % respectively.

2 — The higher the number of bacteria in raw milk the more numerous were the bacteria which survive heating at 72° and 76°C for 15 sec. This relationship was not observed in milk heated at 84°C for 15 sec.

3 — The temperatures of milk pasteurization used in the experiments destroyed 99.7, 99.9 and 100 % of the acid forming bacteria, 88.4, 92.9 and 99.8 % of the caseolytic bacteria, 22.1, 42.4 and 77.1 % of the heat-resistant bacteria.

4 — Among the heat-resistant bacteria in milk, there were observed bacteria belonging to genus *Bacillus*, 60.4 %, *Microbacterium*, 24.6 %, *Micrococcus*, 8 % and other rods and cocci, 7 %, which were not identified.

Ö Z E T

Bu çalışmada, laboratuvar koşullarında çiğ süte uygulanan farklı üç ısı işleminin (72°, 76°, 84°C lerde 15 saniye) mikroflora üzerindeki etkisi incelenmiştir.

Elde edilen sonuçlara göre :

1 — Isı işlemlerinden sonra sütlerin toplam bakteri içeriği uygulanan ısı işlemine göre farklı olmakla birlikte, önemli ölçüde azalmıştır. Toplam bakteri sayısındaki azalma oranları ortalama olarak 72°C de % 96,8, 76°C de % 99,3 ve 84°C de % 99,91 olarak saptanmıştır.

2 — Isı işlemleri sonunda bakteri sayılarında saptanan azalma oranları, çiğ sütün bakteri içeriğine ve mikrofloradaki bakteri türlerine göre değişmiştir. Farklı bakteri türlerinin azalma oranları ısıya dayanıklılıklarına göre değişik bulunmuştur.

3 — Isıya dayanıklı bakteri sayıları yüksek olan çiğ sütlerin 72°C de ve 76°C de 15 saniye tutulmasıyla elde edilen sütlerde de ısıya dayanıklı bakteri sayısı yüksek bulunmuştur. Fakat 84°C de 15 saniye tutulanlarda bu ilişki gözlenmemiştir.

4 — Çiğ sütün 72°, 76° ve 84°C lerde 15 saniye tutulması sonucunda (sırasıyla ve

ortalama olarak) asit yapan bakteri sayısında, % 99,7, % 99,9 ve % 100, Caseolytic bakteri sayısında, % 88,4, % 92,9 ve % 99,3, ısıya dayanıklı bakteri sayısında da % 22,1, % 42,4 ve % 77,1 oranlarında azalma saptanmıştır.

5 — Sütlerden izole edilen, ısıya daya-

nıklı bakterilerin büyük bölümünü, *Bacillus*, *Microbakterium* ve *Micrococcus* cinsleri oluşturmuştur. Bunların toplam izole edilen (Çiğ ve ısı işlemi uygulanmış sütlerden) bakteriler içindeki oranları, sırasıyla % 60,4, % 24,6 ve % 8 diğerlerinin ise % 7 bulunmuştur.

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