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RESEARCH ON THE MICROBIOLOGICAL QUALITY OF THE PASTEURIZED MILK PRESENTING TO THE CONSUMPTION ON ANKARA

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SUMMARY

In this research, totally 54 samples of bottled and cartoned pasteurized milk from different markets in Ankara were examined in order to determine the microbiological quality. There were 10^2-10^3 total viable bacteria per ml in 37% of samples, 10^2-10^3 coliform per ml in 24%, 10^2-10^3 *E.coli* per ml in 7.4%, 10^2-10^3 psychrotrophic microorganisms per ml in 31.4%, $2.0x10^1$ *S.aureus* per ml in 22.2 %, $2.0x10^1$ *Enterococcus* per ml in 9.52% of samples. The results of the survey indicate that the problems are related to poor hygiene during production. In addition to, bottled and cartoned pasteurized milk samples have got risk for the public health and haven't got a good bacteriological quality.

Key words: Pasteurized milk, bacteriological quality

ANKARA'DA TÜKETİLEN PASTÖRİZE SÜTLERİN MİKROBİYOLOJİK KALİTESİ

ÖZET

Bu çalışmada, Ankara'da değişik marketlerden toplanan 54 adet kutu ve pastörize süt örnekleri mikrobiyolojik kaliteleri yönünden incelenmiştir. Toplam canlı bakterinin %37'sinin, koliform'un %24'ünde, *E.coli*' nin %7.4'ünde, psikrotrofik mikroorganizmaların %31.4'ünde tespit edilen koloni sayımı 10^2-10^3 adet/ml iken, *S.aureus*' un %22.2'sinde, Enterokok'un %9.52' sinde tespit edilen koloni sayımı $2.0x10^1$ adet/ml olarak bulunmuştur. Araştırma sonuçları, üretim sırasındaki hijyen koşullarının kötü olduğunu göstermektedir. Buna ilave olarak, şişe ve kutu pastörize süt örneklerinin iyi bir bakteriyolojik kaliteye sahip olmadıkları ve halk sağlığı açısından riske sahip olduğu belirlenmiştir.

Anahtar kelimeler: Pastörize süt, bakteriyolojik kalite

INTRODUCTION

Many pathogen microorganisms (*E.coli, S.aureus, B.cereus, Streptococcus spp*) may be present in pasteurized milk. They may be derived from faeces or symptomless cows, human sources, poor and contaminated environment or dairy equipment. They can be cause of food poisoning especially for the young and the very old, and these are called oppurtunist pathogens. They include types formerly regarded only as indicator or undesirable organisms, such as enteropathogenic *E.coli, S.aureus, B.cereus, coliform, Streptococcus spp, P.aeruginosa*, which

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have been shown during the last 30 years to be capable of causing food poisoning or enteric infections, especially in babies, and sometimes with fatal consequences. Even though pasteurization eliminates the pathogens and most of the psychrophilic microorganisms surviving thermophilic and thermoduric bacteria spoil the milk (1,2). Gram negative rods are killed by pasteurisation, but psychrophilic gram negative rods (especially *Pseudomonas*) are only low level post-pasteurization contaminants. Psychrotrophic microorganisms are defined as those capable of growing at low temperatures (2 to 70˚C) and the ability of the organisms to cause an organoleptically detectable change in pasteurized milk (3). Thus, the objective of this study was to determine kinds of contaminating microorganisms present in cartoned and bottled pasteurized milk.

MATERIAL AND METHODS

In this research, a total 54 samples of bottled and cartoned pasteurized milk from different markets in Ankara were collected over the period May to October 1999. Among the 54 samples, 27 samples were cartoned pasteurized milk, 27 samples were bottled pasteurized milk,respectively. Upon arrival at our laboratory, the bottled and cartoned pasteurized milk were immediately tested.

Preparing the samples for the microbiological analysis

10 ml.samples were homogenized in 90 ml. 1 % steril peptone water. Then up to 10^{-4} dilutions were prepared.

Microbiological analysis

The total viable and psychrotrophic bacteria were enumerated by using Plate Count Agar (Difco) and incubating the plates at 30˚C for 48 h. and 7˚C for 10 days, respectively. Those showing between 30-300 colonies were counted (4).

Enumeration of *E.coli* by MPN (Most Propable Number) technique was carried out in Fluorocult broth (Merck, Germany) using a Durham tube, which was incubated at 37˚C for 16-24 h. Further , the tubes were examined for fluorescence by holding them under ultraviolet lamp (366 nm). Tubes showing fluorescense and gas formation were considered to contain *E.coli* (5). Fluorocult broth and brillant green bile (BGB) were used for MPN evaluation of in the presumptive and confirmation tests for detecting coliforms, as described by Chen (5).

For coagulase positive staphylococcus enumeration, serial dilutions of bottled and cartoned pasteurized milk homogenates were plated on Baird-Parker (Difco) and incubated at 37˚C for 48 h. Typical colonies were confirmed by using Staphaurex rapid test kit (5).

Enterococcus enumeration, 0.1 ml of the diluted sample was plated on to Slanetz and Bartley Agar (SBA). From each SBA plate, typical red colonies were isolated and named as enterococcus (6).

RESULTS

The microbial evaluation and distribution of bottled and cartoned pasteurized 54 milk samples are presented in Table 1.

Regarding the distribution of microbial population, 37% of the samples were noted to have more than 10^2-10^3 cfu/ml of total viable bacteria counts. The largest range of microbial distribution for psychrotrophs was found between 10^2-10^3 cfu/ml, with the detection rate of 31.4%. Coliform counts were mostly found the highest range of 10^2-10^3 cfu/ml, that had a detection rate of 24% in 54 samples. *E.coli* was detected in 7.4% of bottled and cartoned pasteurized 54 milk samples. *S.aureus*, enterococcus, yeast and molds were mostly found highest range of $2.0x10^1$ cfu/ml, that had a detection rates of 22.2%, 9.52%, 24.0%. The detection rates of coliforms, *S.aureus*, enterococcus were 55.5%, 61.1%, 0.14%, respectively. The contamination level of *S.aureus* was higher than that of coliforms and enterococcus in samples (Table 1).

Table 1. Microbial evaluation and distribution of bottled and cartoned pasteurized milk samples[a]

Microorganisms[b]	No of samples in the following range				
	$<2.0x10^1$	$2.0x10^1$	10^2-10^3	10^3-10^4	Incidence (%)
Total viable bacteria count	1 (1.85)[c]	6 (11.1)	20 (37.0)	13 (24.0)	74.0
Psychrotrophic count	6 (11.1)	9 (16.6)	17 (31.4)	2 (3.70)	62.9
Coliforms	2 (3.70)	10 (18.5)	13 (24.0)	5 (9.25)	55.5
S.aureus	8 (14.8)	12 (22.2)	9 (16.6)	4 (7.40)	61.1
Enterococcus	0 (0.0)	5 (9.52)	2 (3.70)	1 (1.85)	0.14

[a] 54 samples were used
[b] Colony forming unit (cfu/ml)
[c] Numbers in parentheses indicate percentage of positive samples

DISCUSSION

The large number of total viable bacteria, contamination indicators (Coliform and *E.coli)*, and pathogens (*S.aureus, Enterococcus*) detected in the pasteurized milk samples surveyed in this investigation revealed that their presence presented a potential health hazard to consumers. In our study, the counts of total viable bacteria, 37% of the pasteurized milk samples were found between 10^2-10^3 cfu/ml and 24% of them were found 10^3-10^4 cfu/ml. Kapadnis and Panse (7) reported that the counts of total viable bacteria and coliform were observed between $1.0x10^5$-$1.0x1.0^9$ cfu/ml, 23-1100 KMS/100 ml. in pasteurized milk. Mahari and Gashe's (8) study were found that of the pasteurized milk samples, 70% had total viable bacteria counts of $\leq 7x10^5$ cfu/ml as it left the pasteurizing unit, but the population increased 2 to 4 fold as a result of subsequent contamination, so Mahari and Gashe's (8) study showed that there were total viable bacteria with $7x10^5$ cfu/ml in the pasteurized milk samples which this result were found higher than our results. Our results of total viable bacteria are not close to the values of Turkish Standart Institution (9) $\leq 4x10^4$ ml and Food Regulations of Turkey (10) 1.class $\leq 2x10^4$/ml, 2. class $\leq 4x10^4$/ml, so in our study, the counts of total viable bacteria shows that hygienic conditions of dairy plants in Ankara were not adequate. Coliform microorganisms especially *E.coli* is a pathogenic bacterium that was responsible for several food related outbreak of disease. Sing and Ranganatham (11) found out that there were $0\text{-}4.5x10^3$ cfu/ml coliforms in the pasteurized cow milk and $0\text{-}1.0x10^4$ cfu/ml coliforms in the pasteurized buffalo milk. In the present study, 38% of the samples $\leq 2.0x10^1$ cfu/ml, 18.5% of them $2.0x10^1$ cfu/ml, 24% of them 10^2-10^3 cfu/ml, 9.25% of them 10^3-10^4 cfu/ml coliform were isolated. In addition to coliforms, *E.coli* were detected in 55.5% and 7.4% of the bottled and cartoned pasteurized milk samples. According to Turkish Standart Institution and Food Regulations of Turkey, coliform counts should not exceed 10 cfu/ml and *E.coli* should not be detected at all in dairy products (9). In addition to, foodborne patogens such as *S.aureus, L.monocytogenes* shouldn't be detected in foods as well.

Psychrotrophs are found invariably in pasteurized milk as a result of post-pasteurisation contamination. Although in a commercial dairy the number of these psychrotrophs in freshly pasteurized milk is in the order of 5 per 100 ml or less, their multiplication during refrigerated storage primarily determines its shelf-life (12). Mahari and Gashe's (8) indicated that the detection rate of psychrotrophs microorganisms was 98.1% in pasteurized milk. Psychrotrophs microorganisms were detected 62.9% bottled and cartoned pasteurized milk samples in present study (Table 1). This indicated that psychrotrophs microorganisms were contaminated after pasteurization and packing process.

Most of economic loss due to mastititis is a consequence of infection with *Staphylococcus aureus*, *Streptococcus agalactiae, Enterococcus or Escherichia coli*. These bacteria are commonly presented in milk samples collected aseptically, but rarely produce clinical mastitis or markedly reduce milk yield. (13). All reported incidents of staphylococcal food poisoning including those caused by milk and milk products, have been attributed to *S.aureus*. Milk and milk products can become contaminated if good hygiene control is not exercised on farms, the milk is inadequately pasteurized and precautions are not taken to prevent contamination and subsequent growth of *S.aureus* during the manufacturing processes and in the finished product (14).

Magdy indicated that $1.04x10^{10}$ cfu/ml enterococcus, $1.30x10^{9}$ cfu/ml coliform, $2.47x10^{5}$ cfu/ml staphylococcus were isolated in pasteurized milk (15). Stanescu and et.al reported that in pasteurized milk, $1.34x10^{5}$ cfu/ml coliform, $2.4x10^{1}$ cfu/ml *E.coli* were isolated (16). A review by Ambarcı showed that the colony counts of total viable bacteria, coliform, staphylococcus and enterococcus were 10^{2}-10^{6} cfu/ml, 0-$6.4x10^{4}$ cfu/ml, 0-10^{5} cfu/ml, 0-$3.1x10^{3}$ cfu/ml in pasteurized milk (17). In our study, the colony counts of *S. aureus, Enterococcus* were found ≤$2.0x10^{1}$ - 10^{4}, $2.0x10^{1}$-10^{4} cfu/ml, respectively. The largest range of microbial distribution for *S.aureus* and *Enterococcus* were found $2.0x20^{1}$ cfu/ml. The result of colony counts of Magdy and Ambarcı's for *S.aureus* was found higher than while *Enterococcus* was found lower than our results (15,17). These results showed that *S.aureus* and *Enterococcus* were found as post-pasteurisation contamination bacteria in bottled and cartoned pasteurized milk.

In summary, it is important to realize that for the production of bacteriological quality pasteurized milk, controlling *S.aureus, coliform, E.coli, Enterococcus* and psychrotrophic contamination and growth is essential. The increase in keeping quality, particularly when refrigeration at 72˚C or less is used, is an extremely important advantage of pasteurisation. Psychrotrophic microorganisms responsible for most defects of refrigerated milk are killed by pasteurization, but *S.aureus* and *Enterococcus*, which can survive after pasteurisation, grow very slowly under good refrigeration, and ordinarily will be cause of defects only after prolonged holding under refrigeration or when the holding temperature has been considerable higher than desirable. When post - pasteurisation contamination can be avoided, pasteurisation markedly extends the time before defects appear.

REFERENCES

1. Thomas SB, The microflora of bulk collected milk. Part 2. Dairy Industries 1974; 39: 279-282.
2. Collins EB, Heat resistant psychrotrophic microorganisms. Dairy Science, 1981; 64: 157-160.
3. Nelson FE, The microbiolgy of market milk. In: Robinson RK. Dairy Microbiolgy, vol 1. Applied Science Publishers. London, 1981; 165-207.
4. Harrigan WF, Mc Cance ME. Laboratory methods in food and dairy microbiology. Academic Press, London, 1976; pp 452 .
5. Chyan AT, Chan JH, Chiou TT and Hu CH. An investigation on *Bacillus cereus*, *Staphylococcus aureus*, coliforms and *Escherichia coli* or rice products in Taiwan. Ann Rept. NLFD Taiwan, ROC, 1989; 7: 131-134.
6. Svec P, Sedlacek I. Occurrence of *Enterococcus spp.* in waters. Folia Microbiology. 1999; 44 (1), 3-10.
7.Kapadnis BP, Panse MV. Bacteriological quality of pasteurized milk from Poona city. Indian J Microbiol. 1986; 26 (3-4): 294-296.
8. Mahari T, Gashe B. A survey of the microflora of raw and pasteurized milk processing plant in Addis Ababa, Ethiopia. J Dairy Research. 1990; 57: 233-238.
9. TSE. Pastörize süt TS 1019. Türk Standartları Enstitüsü, Ankara 1988.
10. Anonim, gıda maddelerinin ve umumi sağlığı ilgilendiren eşya ve levazımın hususi vasıflarını gösteren tüzük. Titiz Ofset Matbaası. Ankara, 1982; 16-17.
11. Sing RS, Ranganathan. Incidence and distribution of *E.coli* in dairy products. Indian J Dairy Sci. 1978; 31 (1): 82-84.
12. Rowe M, Gilmour A. The present and future importance of psychrotrophic bacteria. Dairy Industries International, 1985; 50 (11).
13. Bramley A J. Infection of the udder with coagulase negative micrococci and *Corynebacterium bovis*. In: Proc.

Sem. on Mastitis Control. Dodd FH, Griffin TK, Kingwill RG. (eds) International Dairy Federation Doc. No 85. Brussels. 1975.
14. Holmerg SD, Blake PA. Staphylococcal food poisoning in the United States. Journal of the American Medical Association, 1984; 251, 487-489.
15. Magdy SE. Bacteriological quality of pasteurized milk in Tripoli city. In: Proceedings of 3rd world Congress of Foodborne Infections and Intoxications, 1992; 189.
16. Stanescu V, Chırıla F, Sahleanuc Vana V, Dawian A. The level of coliform and *E.coli* titre as hygienic quality index of raw and pasteurized milk of chicken meat. In: Proceeding of 3rd World Congress of Foodborne Infections and Intoxications, Berlin, 1992; 351-354.
17. Ambarcı I. Ankara piyasasında satılan pastörize sütlerin mikrobiyolojik nitelikleri üzerinde araştırmalar. Bornova Vet. Araşt. Enst. Derg. 1972; 24 (13): 4-49.