The Effect of Freezing on Different Bacterial Counts in Raw Milk

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Abstract

This study had investigated the effect of freezing on the bacterial counts in raw milk. In specific, we estimated the total bacterial counts and the counts of total coliform, Salt tolerant bacteria, *Staphylococcus spp*., Thermopilic Lactobacilli, Mesophilic Lactobacilli, *Leuconostoc spp.,* Lactococci and Streptococci. Thirty samples of raw cow milk were frozen at -18-20°C for eight weeks and tested on a weekly basis. The results showed that the bacterial counts had significantly decreased as the freezing period increased. Thus, the total count had significantly decreased from ($6.5*10^5$ cfu/ml) in fresh milk to ($5.0*10^3$ cfu/ml) in frozen milk after eight weeks ($P \leq 1\%$). The decrease in the bacterial counts was different between the different bacterial groups. The highest decrease was for Salt tolerant bacteria from ($9.8*10^5$ cfu/ml) to ($7.6*10^3$ cfu/ml), while the lowest decrease was for *Staphylococcus spp* from ($3.6*10^3$cfu/ml) to ($8.2*10^1$cfu/ml). The study recommends freezing as an important storage method for raw milk.

Keywords: raw milk, freezing, bacterial count, food storage.

1. Introduction

Milk is considered as a suitable media for microbial growth for several reasons, it is a rich source of nutrients, the pH of the milk is approximately neutral and its water content is high. Freezing storage is a strategy used to overcome the concern regarding seasonality of food of animal origin and improve handling, preservation and marketing of agricultural productions (Hundy et al., 2008). Freezing does not sterilize food. The extreme cold simply delays the growth of microorganisms and slows down the changes that affect quality or cause spoilage in food. Several studies had investigated freezing as a technique for storing foods and decreasing microorganisms (Storper et al., 1982; Pankey et al., 1987; Schukken et al., 1989). Schukken et al. (1989) demonstrated a decrease in the number of samples positive for *Escherichia coli* and *Arcanobacterium pyogenes* and an increase in the number of coagulase negative staphylococci (CoNS) positive samples after freezing at -20 °C and storage for four, eight and sixteen weeks. On the other hand, Villanueva et al. (1991) documented that after a period of frozen storage of milk samples at -20°C, the frequency of *Streptococcus agalactiae* isolation increased 2.50 times. The frequency of *Staphylococcus aureus* isolation increased 1.48 times in the same interval. Sol et al. (2002) evaluated three techniques of isolation of pathogenic microorganisms :(1) a conventional culture technique (IDF 1981); (2) incubation of milk in broth, followed by a conventional culture technique; (3) freezing the whole milk sample, followed by incubation using a conventional culture technique. They found that a combination of standard culture technique and freezing plus incubation was most attractive for achieving a high isolation rate of *Streptococcus agalactiae* and *Streptococcus dysgalactiae*. Sanchez et al. (2003) studied the effect of freezing on goat milk. Unlike cow milk, they did not find significant decrease in *E. coli* count in goat milk samples after freezing at -20 °C, and even after 730 days of storage at -80 °C. These results were explained by the differences in milk composition. Hubackova and Rysanek (2007) examined the effects of freezing and subsequent storage on quantitative results of bacteriologic culturing of selected alimentary pathogens and indicator microorganisms in milk. They documented that freezing had a considerable adverse effect on the recovery of *E. coli* after 7 days of storage. A decline in counts of coliform bacteria and psychrotrophic microorganisms was detected as soon as after 72 hours storage. The decline in counts of *E. coli* and psychrotrophic microorganisms continued with the time of storage 72 hours and 7 days; 72 hours and 21 days. On the contrary, no effect was recorded for *Listeria* monocytogenes and total bacterial count. Freezing caused a slight increase in *Staphylococcus aureus* counts in milk samples after 72 hours and 7 days of storage. Pazzola et al. (2013) investigated the effect of long-term

freezing on milk renneting properties from the Sarda sheep, an autochthonous breed from Italy. They concluded that the remarkable decreasing of sheep milk renneting characteristics after frozen storage can predict a worse yield and quality of cheese-making and suggests that freezing of Sarda raw milk should be limited to shorter periods. Nurliyani et al. (2015) investigated the change of milk quality and emulsion stability during 60 d frozen storage. Milk sample was taken from three Ettawah Crossed bred goats that were divided into three groups. Samples were frozen and stored for 0, 30 and 60 days. Thereafter, the microbiological and chemical data were analyzed. Authors found no change in the total bacteria, acidity, pH and free fatty acid of milk during storage, whereas the assessment by 70% alcohol showed positive since 0 days. Emulsions stability changed after 30 days of storage. While, the clot on boiling test of milk was positive at 60 days of storage. The study recommended that frozen storage of goat milk should not longer than 30 days. Given the mixed evidence of the effect of freezing on the bacterial growth in milk, this study reexamined this effect however, using different groups of bacteria and using specific media for enumerating each. The remaining of the study is organized as follows: Section 2 describes materials and methods. Section 3 discusses the results of the study. Section 4 concludes.

2. Materials and Methods

Milk samples were collected from cow milk from different areas in Jordan and tested for the effect of freezing at -20°C in order to explore the behavior of different bacterial groups. We chose the following bacterial groups because they are more frequently found in milk than others. The test period ranged from one to eight weeks.

2.1 Microbiological Analysis:

2.1.1 Enumeration of Microbial Groups on Different Media:

Serial dilutions from milk samples were prepared in sterile 1% (w/v) peptone water plated on selective agar and incubated at the appropriate temperatures.

2.1.2 Total Bacterial Counts

The total bacterial counts were enumerated on (PCA) Plate count agar (Difco); plates were incubated for 48h at 32˚C, (Difco, 1984).

2.1.3 Lactococci and Streptococci

For counting the lactococci and streptococci groups, the M17 (Terzaghi and Sandine, 1975) medium (Difco) was used; plates were incubated for 48h at 30 and 42 ˚C respectively.

2.1.4 Mesophilic and Thermophilic Lactobacilli

The MRS (De Man et al., 1960) agar medium (Biolife) was used after acidifying to PH 5.2-5.4 to enumerate the mesophilic and thermophilic lactobacilli. The plates were incubated anaerobically for 72 h at 30 and 42 ˚C respectively.

2.1.5 Counts of Leuconostoc spp.

The MSE (Mayeux et al., 1962) medium was used for counting the *Leuconostoc* spp.; plates were incubated at 30˚C for 48h.

2.1.6 Total Coliform Bacteria

For counting the total coliform bacteria, the VRBA (violet red bile agar) medium (Difco) was used as recommended by the Standard Methods for the Examination of Dairy Product (1960); plates were incubated at 37˚C for 48h.

2.1.7 Staphylococci

The MSA (manitol salt agar) medium (Biolife) were used to enumerate the total staphylococci; plates were incubated at 37˚C for 48h.

2.1.8 Salt-Tolerant Bacteria

The BHI (brain heart infusion) agar medium (Difco) supplemented with 5% (w/v) NaCl were used to count the salt-tolerant bacteria; plates were incubated at 25˚C for 48h, (Difco, 1984).

2.2 Statistical Analysis

To accomplish the Gaussian data distribution, we performed simple logarithmic transformation (with logarithms base 10). CFU/ml counts were statistically evaluated after logarithmic transformation using one-level analysis of variability and by the assessment of significance of differences using the Tukey method (NIST/SEMATECH 2005). The data was processed by STAT Plus software (Matouskova et al., 1992).

3. Results and Discussion

Table 1 reports the results of the microbial analysis of the milk samples. The results indicated that the total bacterial count had significantly (P≤1%) decreased from (6.5*10^5 CFU/ml) in fresh milk to (5.0*10^3 CFU/ml) after eight weeks of freezing. The decrease was gradual over the weeks one to eight. Freezing caused a statistically significant decrease (P ≤ 0.01) in CFU/ml counts of Salt tolerant bacteria. In specific, it had decreases from (9.8*10^5 CFU/ml) in fresh milk to (7.6*10^3 CFU/ml) at the end of the freezing period. Thermopilic and Mesophilic Lactobacilli had significantly (P ≤ 0.01) decreased from (8.5*10^4 CFU/ml) to (1.1*10^3 CFU/ml) and from (7.1*10^3 CFU/ml) to(7.1*10^2 CFU/ml), respectively. Similarly, Streptococci and Lactococci had significantly (P ≤ 0.01) decreased from (1.6*10^5 CFU/ml) to (1.4*10^3 CFU/ml) and from (1.8*10^5 CFU/ml) to (1.1*10^3 CFU/ml), respectively. Freezing resulted in a statistically significant decrease (P ≤ 0.01) in CFU/ml counts of *Leuconostoc spp*. On the other hand, Total coliform and *Staphylococcus* spp showed no statistically significant (P ≤ 0.01) decrease after the freezing period.

These results are consistent with those of (Schukken et al., 1989; Hubackova & Rysanek, 2007) who documented a statistically significant decrease of *E. coli* after freezing. Hubackova and Rysanek (2007) also found a minor increase in *Staphylococcus aureus* counts in milk samples after 72 hours and 7 days of storage which again supports our results. However, our results are contrasting with (Nurliyani et al., 2015) who found no change in the total bacteria in milk samples which were frozen and stored for 0, 30 and 60 days. Overall, our findings indicated that freezing is a good way for storing dairy products and specifically milk. It is a vital technique to control the increase in the bacterial counts and thus storing milk for longer periods comparing to other methods such as cooling. These findings have important implications for microbiologists and manufacturers of milk products.

Table 1. The Microbial Analysis

Bacterial count cfu/ml

Bold values are statistically significant at $p \leq 1\%$

Media	Target strains	Fresh milk	After 1 week	After 2 week	After 3 week	After 4 week	After 5 week	After 6 week	After 7 week	After 8 week
PCA	Total count	6.5*10^5	6.1*10^5	5.4*10^5	3.4*10^5	**6.3*10^4**	**1.5*10^4**	**6.3*10^3**	**5.3*10^3**	**5.0*10^3**
VRBA	Total coliform	5.9*10^3	5.3*10^3	4.3*10^3	2.9*10^3	8.4*10^2	3.4*10^2	1.4*10^2	6.8*10^1	5.4*10^1
BHI+5%salt	Salt tolerant bacteria	9.8*10^5	9.5*10^5	8.8*10^5	6.2*10^5	2.4*10^5	**8.1*10^4**	**8.4*10^3**	**8.0*10^3**	**7.6*10^3**
MSA	*Staphylococcus spp*	3.6*10^3	3.2*10^3	2.0*10^3	1.8*10^3	6.8*10^2	2.1*10^2	1.1*10^2	8.5*10^1	8.2*10^1
MRS(42°C)	Thermopilic Lactobacilli	8.5*10^4	8.2*10^4	6.8*10^4	5.4*10^4	1.2*10^4	**7.6*10^3**	**1.4*10^3**	**1.1*10^3**	**1.1*10^3**
MRS(32°C)	Mesophilic Lactobacilli	7.1*10^3	6.6*10^3	5.9*10^3	4.6*10^3	1.1*10^3	**8.5*10^2**	**3.2*10^2**	**7.6*10^2**	**7.1*10^2**
M17(42°C)	Streptococci	1.6*10^5	1.1*10^5	1.0*10^5	**8.5*10^4**	**3.1*10^4**	**5.9*10^3**	**2.4*10^3**	**1.8*10^3**	**1.4*10^3**
M17(32°C)	Lactococci	1.8*10^5	1.2*10^5	**9.7*10^4**	**7.4*10^4**	**1.2*10^4**	**6.1*10^3**	**2.1*10^3**	**1.4*10^3**	**1.1*10^3**
MSE	*Leuconostoc spp.*	6.5*10^4	6.4*10^4	6.0*10^4	**5.2*10^3**	**4.0*10^3**	**7.8*10^2**	**2.8*10^2**	**1.7*10^2**	**1.6*10^2**

4. Conclusions

The objective of the present study was to determine the effects of freezing on the counts of different bacterial groups including (total count, total coliform, Salt tolerant bacteria, *Staphylococcus spp*., Thermopilic Lactobacilli, Mesophilic Lactobacilli, *Leuconostoc spp.,* Lactococci and Streptococci.) in milk samples collected from different areas in Jordan. We froze the samples at 20°C for periods ranging from one to eight weeks. The results showed statistically significant decreases in the total bacterial count and the counts of Salt tolerant Thermopilic Lactobacilli, Mesophilic Lactobacilli, *Leuconostoc spp.,* Lactococci and Streptococci bacteria; On the contrary, no

statistically significant decrease was noticed in the counts of Total coliform and *Staphylococcus spp* after eight weeks of freezing. These results are important for the topic of food safety and storage.

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