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# MICROBIOLOGICAL QUALITY OF FRESH AND HEAT TREATED COW'S MILK DURING STORAGE

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## ABSTRACT
The aim of the present study was to evaluate the microbiological quality of raw milk from milk vending machine and heat treated milk during storage. There were analyzed 120 samples of milk (30 samples of fresh milk, 30 samples of raw milk stored 4 day at 4 °C, 30 samples of heat treated milk – 70 °C stored 4 day at 4 °C and 30 samples of heat treated milk – 100 °C stored 4 day at 4 °C). Total viable counts (TVC), coliform bacteria (CB) and microscopic filamentous fungi (MFF) were determined by microbiological analysis. Plate dilution method were used for microbiological analysis. The number of total viable counts (TVC) in fresh milk ranged from 4.08 log KTJ.mL$^{-1}$ to 4.89 CFU.mL$^{-1}$. TVC in raw milk after storage ranged from 5.31 log CFU.mL$^{-1}$ to 6.81 log CFU.mL$^{-1}$. TVC in heat treated milk with temperature 70 °C after storage ranged from 3.89 log CFU.mL$^{-1}$ to 4.45 log CFU.mL$^{-1}$ and TVC in heat treated milk with temperature 100 °C after storage ranged from 2.96 log KTJ.mL$^{-1}$ to 3.91 log KTJ.mL$^{-1}$ in heat treated milk with temperature 100 °C after storage. The number of CB were in range from 1.49 log CFU.mL$^{-1}$ to 1.89 log CFU.mL$^{-1}$ in fresh milk, from 1.99 log CFU.mL$^{-1}$ to 2.61 log CFU.mL$^{-1}$ in raw stored milk. Coliform bacteria were not present in heat-treated milk samples. The values of MFF ranged from 0 log CFU.mL$^{-1}$ to 2.01 log CFU.mL$^{-1}$ in fresh milk, from 1.43 log CFU.mL$^{-1}$ to 3.98 log CFU.mL$^{-1}$ in raw milk after storage, from 1.33 log CFU.mL$^{-1}$ to 3.41 log CFU.mL$^{-1}$ in heat treated milk with temperature 70 °C after storage and from 1.30 log CFU.mL$^{-1}$ to 3.32 log CFU.mL$^{-1}$ in heat treated milk with temperature 100 °C after storage.

**Keywords:** milk; total viable counts; coliform bacteria; microscopic filamentous fungi; heat treatment

## INTRODUCTION
Milk and dairy products are important components of the diet worldwide. The quality and shelf life of liquid milk as well as dairy products are often compromised by flavor, odors, and visual defects arising from the bacterial growth and activities of heat-stable enzymes produced by psychrotrophic bacteria before processing **(Techer et al., 2014)**.

Milk testing and quality control should be carried out at all stages of the dairy chain **(Zajác et al., 2015)**.

The quality and safety of raw cow's milk is very important for dairy companies and consumers of milk products. Due to the methods of production, it is impossible to completely eliminate contamination of milk with microorganisms, therefore the microbial content of milk is a major feature in determining its quality **(Zajác et al., 2012)**.

Milk is a nutritious food for humans but it is also an ideal growth medium for bacterial pathogens **(Ruusunen et al., 2013)**. The consumption of raw milk is not well-documented, but in the context of the current trend toward "consuming natural", consumption of raw milk is becoming more popular. However, due to its high nutritional value together with the neutral pH and high water activity, raw milk is good growth medium for different micro-organisms, whose multiplication depends mainly on temperature and on competing micro-organisms and their metabolic products. In order to guarantee its microbial safety and to prolong its shelf-life, milk is heat treated **(Claeys et al., 2013)**.

In general, raw milk intended for human consumption must meet the requirements of the General Food Law **(Regulation (EC) 178/2002)** and be free of pathogens.

Raw milk can be a source of pathogenic bacteria. Bacteria can get into milk directly from cow breeding or in the non-hygienic handling of fresh raw milk. Consumption of untreated raw milk may pose a risk to humans. **(Wouters et al., 2002)**. The use of hygienic milking procedures and hygienic storage are the most importance in reducing the levels of contamination of milk by microorganisms **(Mhone et al., 2011)**.

The total viable counts in milk has a decisive effect on the quality and safety of dairy products **(Szteyn et al., 2005)**. Milk contaminated by high levels of spoilage bacteria usually becomes unsuitable for further processing since it does not meet the consumer's expectations in terms of health, safety and satisfaction **(Nanu et al., 2007)**. The presence of total coliforms in food of animal origin shows to environmental sources of contamination since these micro-organisms are abundant in the environment **(Shojaei and Yadollahi, 2008)**. *Escherichia coli* is the most common contaminant of raw and processed milk. It is a reliable indicator of faecal contamination of water and food **(Todar, 2008)**. *E. coli* is a commensal micro-organism of the intestines of animals and humans but its presence in food may be of public health concern due to the possible presence of enteropathogenic and/or toxigenic strains **(Soomro et al., 2002)**.

Fungi are eukaryotic, Gram positive, non-acid fast, heterophilic, non-photosynthetic, osmotrophic and saprobic microorganisms. Presently, over 250,000 fungi are present in our environment. The fungi are ubiquitous in distribution, and are found in the soil, water, and air **(Pal, 2007)**.The fungi which include moulds and yeasts are responsible for the spoilage of milk and milk products **(Pal and Jadhav,2013)**.

The fungal contamination of dairy products can occur from environment, equipments, handlers and packaging materials. Among these fungi, *Aspergillus* ,*Fusarium* and *Penicillium* are important as they produce mycotoxins which can cause serious health hazards. In order to detect the source of fungal contamination in milk products, molecular tools should be applied in the dairy enterprises. The application of hazard analysis control point (HACCP), good manufacturing practice (GMP), sanitation, and preservation of open milk products in refrigeration can avoid the contamination of the dairy products from fungi, and thereby, prevent the economic loss in dairy establishment **(Pal, 2014)**.

## Scientific hypothesis

The microbiological quality of the raw milk and heat treated milk during storage at temperature 4 °C was compared. It is prerequisite, that the raw milk contains undesirable microorganisms, so it can be dangerous for human consumption. The heat treatment to 70 °C during 5 minutes were used, because it's assumed, that most of bacteria are eliminated at this temperature and 100 °C during 5 seconds were used because many consumers boil raw milk before consumption.

## MATERIAL AND METHODOLOGY

Microbiological quality of raw milk from milk vending machine and heat treated milk was evaluated in this study. There were analyzed 120 samples of milk (30 samples of fresh milk, 30 samples of raw milk stored 4 day at 4 °C, 30 samples of heat treated milk – 70 °C stored 4 day at 4 °C and 30 samples of heat treated milk – 100 °C stored 4 day at 4 °C. The heat treated milk samples were heated in water bath to 70 °C during 30 seconds and to 100 °C during 10 seconds. Total viable counts (TVC), coliform bacteria (CB) and microscopic filamentous fungi (MFF) were examinated. Plate dilution method was used for quantitative cfu counts of groups of microorganisms in 1 ml of milk. Gelatinous nutritive substrate in petri dishes was inoculated with 1 ml of milk samples by flushing and on surface in three replications. Basic dilutions ($10^{-1}$) was obtained by mixing 5 g of the sample (cheese) and 45 ml of physiological solution (0.85% NaCl). Plate Count Agar (PCA, Oxoid, UK) was used for determine of TVC in samples. Dilutions of $10^{-3}$ and $10^{-4}$ were used to determine of TVC. Petri dishes were cultivated upside-down in a thermostat at 30 °C for 48 – 72 hours (STN EN ISO 4833).

Violet red bile agar (VRBA, Oxoid, UK) was used for determine of CB in samples. Dilutions of $10^{-1}$ and $10^{-2}$ were used to determine of CB. Petri dishes were cultivated upside-down in a thermostat at 37 °C for 24 – 48 hours (STN EN ISO 4832). Dichloran Rose-Bengal Chloramphenicol (DRBC, Oxoid, UK). Agar was used for determine of MFF. Dilutions of $10^{-1}$ and $10^{-2}$ were used to determine of MFF. Petri dishes were cultivated upside-down in a thermostat at 25 °C for 5 – 7 days (STN ISO 7954). The heat treatment of milk and preparing of milk samples were performed in accordance with ISO 6887-5.

### Calculation of microorganisms

The number of microorganisms in1 g samples (N) were calculated using the following formula:

$$\mathbf{N = \Sigma C / [(n_1 + 0.1n_2) \times d]}$$

**ΣC** – sum of characteristic colonies on selected plates,
$\mathbf{n_1}$ – number of dishes from 1. dilutions used to calculate,
$\mathbf{n_2}$– number of dishes from 2. dilutions used to calculate,
**d** – dilution factor identical with 1. used dilution.

### Statisic analysis

Mathematical and statistical analyzes are processed in the tables. Arithmetic mean, standard deviation, coefficient of variation (%) were performed using Excel.
Statistically significant differences by paired t-test between tested parameters were performed in program Tanagra v 1.4.50.

## RESULTS AND DISCUSSION

The sale of raw milk in large amounts directly to consumers through vending machines or via other means has increased in recent years in many countries **(Bianchi et al., 2013)**. Studies have shown, that many consumers prefer raw milk due to better taste and believe in better nutritional value and several health benefits compared to heat treated milk **(Claeys et al., 2013)**.

In Europe, the current regulatory microbial criteria for raw cow milk are ≤100 000 CFU.mL$^{-1}$ (5 log CFU.mL$^{-1}$) for total viable counts (at 30 °C) **(Regulation (EC) 853/2004)**.

The average values of TVC in milk samples are shown in the Table 1. The number of TVC ranged from 4.08 log CFU.mL$^{-1}$ to 4.89 CFU.mL$^{-1}$ in fresh milk, from 5.31 log CFU.mL$^{-1}$ to 6.81 log CFU.mL$^{-1}$ in raw milk after storage, from 3.89 log CFU.mL$^{-1}$ to 4.45 log CFU.mL$^{-1}$ in heat treated milk with temperature 70 °C after storage and from 2.96 log CFU.mL$^{-1}$ to 3.91 log CFU.mL$^{-1}$ in heat treated milk with temperature 100 °C after storage (Figure 1). The TVC in samples of fresh milk were in accordance with Regulation (EC) 853/2004. The values of TVC were significantly higher ($p$ <0.001) in samples of raw stored

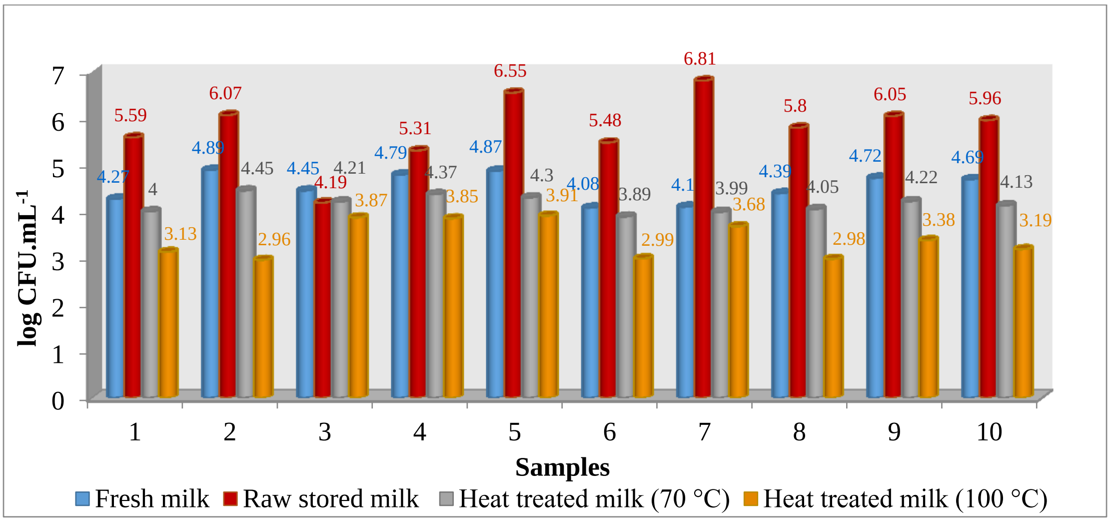


**Figure 1** Total viable counts values in milk.

**Table 1** Basic statistical characteristics of TVC in milk.

| | n | x | s | min | max | V% |
|---|---|---|---|---|---|---|
| **Fresh milk** | 10 | 4.53 | 0.29 | 4.08 | 4.89 | 6.40 |
| **Raw stored milk** | 10 | 5.98 | 0.44 | 5.31 | 6.81 | 7.36 |
| **Heat treated milk (70 °C)** | 10 | 4.16 | 0.17 | 3.89 | 4.45 | 4.09 |
| **Heat treated milk (100 °C)** | 10 | 3.39 | 0.38 | 2.96 | 3.91 | 11.21 |

n – number of samples, x – average, s – standard deviation, v% – coefficient of variation.

milk in comparison with fresh milk and heat treated milk. **Lan et al. (2017)** analyzed 160 raw milk samples, the TVC varied from 3.15 to 6.61 log CFU.mL$^{-1}$, with the average of 5.10 log CFU.mL$^{-1}$. **Kalmus et al. (2015)** examined raw milk from 14 dairy farms for TVC. The total bacterial counts exceeded 100 000 CFU.mL$^{-1}$ in three (21.4%) bulk milk samples and in 10 samples (71.4%) collected at the retail level.

In the study of **Vietoris et al (2016)** the bacteriological quality of raw cow's milk sold in vending machines was evaluated. They found that 64 out of 70 samples (91%) of raw cow's milk from the milk vending machines coincided with the criterion of maximum value of TBC 100 000 CFU.mL$^{-1}$ (5.00 log10 CFU.mL$^{-1}$) according the European Commission Regulation No. 1662/2006. They have found the average value of total bacterial count in bulk tank raw cow's milk samples 4.61 log10 CFU.mL$^{-1}$ and average value of total bacterial count in vending machine raw cow's milk samples 4.76 log10 CFU.mL$^{-1}$.

The risk caused by consumption of raw milk is reduced and even eliminated by a heat treatment. Based on the temperature and time applied, different heat treatments can be used, such as thermization, pasteurization and

**Table 2** Basic statistical characteristics of CB in milk.

| | n | x | s | min | max | V% |
|---|---|---|---|---|---|---|
| **Fresh milk** | 10 | 1.67 | 0.14 | 1.49 | 1.89 | 8.38 |
| **Raw stored milk** | 10 | 2.32 | 0.19 | 1.99 | 2.61 | 8.19 |
| **Heat treated milk (70 °C)** | 10 | 0 | 0 | 0 | 0 | 0 |
| **Heat treated milk (100 °C)** | 10 | 0 | 0 | 0 | 0 | 0 |

n – number of samples, x – average, s – standard deviation, v% – coefficient of variation.

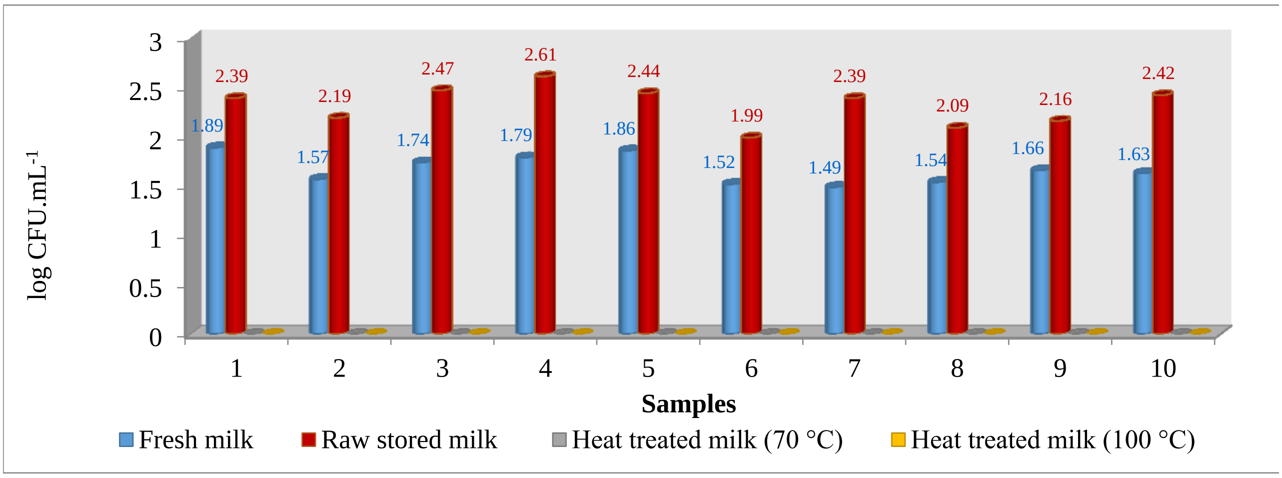


**Figure 2** Values of coliform bacteria in milk.

**Table 3** Basic statistical characteristics of MFF in milk.

| | n | x | s | min | max | V% |
|---|---|---|---|---|---|---|
| **Fresh milk** | 10 | 1.26 | 0.85 | 0 | 2.01 | 67.46 |
| **Raw stored milk** | 10 | 2.78 | 0.73 | 1.43 | 3.98 | 26.26 |
| **Heat treated milk (70 °C)** | 10 | 2.50 | 0.62 | 1.33 | 3.41 | 24.80 |
| **Heat treated milk (100 °C)** | 10 | 2.38 | 0.63 | 1.30 | 3.32 | 26.47 |

n – number of samples, x – average, s – standard deviation, v% – coefficient of variation.

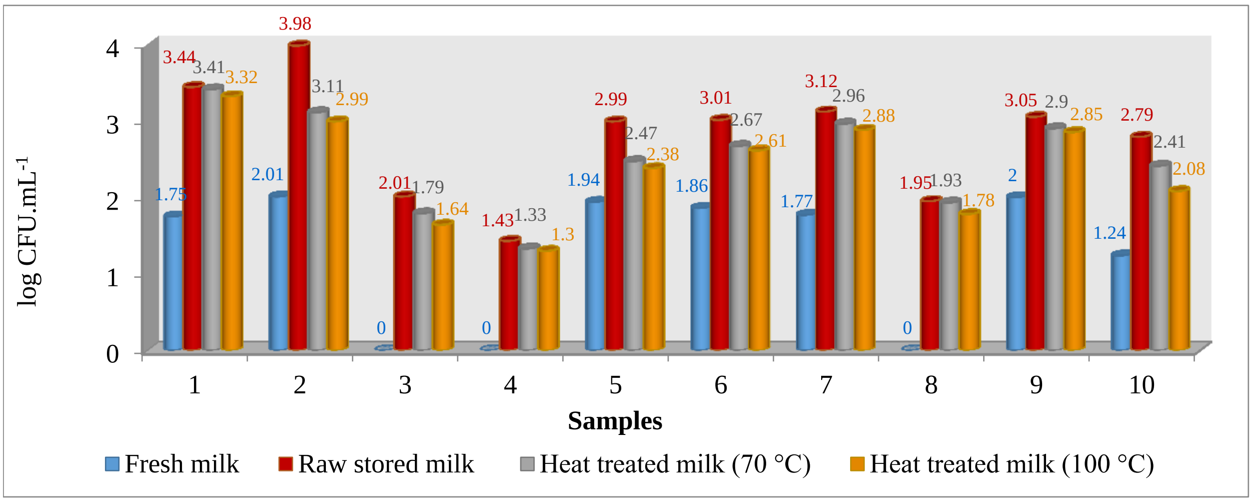


**Figure 3** Values of microscopic filamentous fungi in milk.

sterilization, including UHT (ultra high temperature) and ISI (innovative steam injection) treatment, aimed at different microbial targets and resulting in a different shelf-life of the milk **(Claeys et al., 2013)**.

In the study of **Tremonte et al. (2014)**, the microbiological quality of 30 raw milk samples from 3 vending machines were examined. Milk samples were stored for 72 h at 4 °C and then subjected to different treatments, such as boiling and microwaving, to simulate domestic handling. The results show that all the raw milk samples examined immediately after their collection were affected by high microbial loads, with values very close to or even greater than those acceptable by european legislation. The microbial populations increased during refrigeration, reaching after 72 h values of about 8.0 log CFU.mL$^{-1}$ for *Pseudomonas* spp., 6.5 log CFU.mL$^{-1}$ for yeasts, and up to 4.0 log CFU.mL$^{-1}$ for *Enterobacteriaceae*. Boiling treatment, applied after 72 h to refrigerated milk samples, caused complete decontamination, but negatively affected the nutritional quality of the milk, as demonstrated by a drastic reduction of whey proteins.

The average values of CB in milk samples are shown in the Table 2. The number of CB were in range from 1.49 log CFU.mL$^{-1}$ to 1.89 log CFU.mL$^{-1}$ in fresh milk, from 1.99 log CFU mL$^{-1}$ to 2.61 log CFU.mL$^{-1}$ in raw stored milk. Coliform bacteria were not present in heat-treated milk samples (Figure 2). The values of CB were significantly lower ($p$ <0.001) in samples of fresh milk and heat treated milk in comparison with raw stored milk.

The prevalence and high level of *E. coli* in food of animal origin implies environmental and fecal contamination **(Mhone et al., 2011)**. Other authors have reported that some herd management practices were associated with *E. coli* contamination, such as milking machine, milking parlor type, and milking hygiene **(Piepers et al., 2014)**. Many microorganisms can get access to milk and products, among these are *E. coli*. Coliforms and *E. coli* are often used as marker organisms. *E. coli* is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which mean a public health hazard. *E. coli* is one of the main inhabitants of the intestinal tract of most mammalian species. Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extraintestinal diseases in man **(Kaper et al., 2004)**.

**Altalhi and Hassan (2009)** analyzed 50 samples of milk from different sources for present of coliform bacteria, 40 samples (80%) were contaminated by coliform bacteria, seven samples (17.5%) with non-faecal coliform bacteria, and 10 (20%) were with no growth. In the study of **Hill et al. (2012)** the presence of *Escherichia coli* in raw milk was examinated, 99% of samples tested for *E. coli* had counts <10$^{2}$ CFU.mL$^{-1}$ and only 0.7% were >10$^{3}$CFU.ml$^{-1}$.

Microscopic filamentous fungi usually present in raw milk do not survive pasteurization. Their presence in pasteurized milk and other milk products is caused by reinfection during manufacturing **(Jodral et al., 1993)**.

The moulds have little practical importance in raw milk, but they are important in pasteurized milk, especially when it is used for the manufacture of cheese and other dairy products **(Wouters et al., 2002)**.

The average values of MFF in milk samples are shown in the Table 3.

The values of MFF ranged from 0 log CFU.mL$^{-1}$ to 2.01 log CFU.mL$^{-1}$ in freah milk, from 1.43 log CFU.mL$^{-1}$ to 3.98 log CFU.mL$^{-1}$ in raw milk after storage, from 1.33 log CFU.mL$^{-1}$ to 3.41 log CFU.mL$^{-1}$ in heat treated milk with temperature 70 °C after storage and from 1.30 log CFU.mL$^{-1}$ to 3.32 log CFU.mL$^{-1}$ in heat treated milk with temperature 100 °C after storage (Figure 3). The values of MFF were significantly lower ($p$ <0.001) in samples of fresh milk in comparison with raw stored milk and heat treated milk.

**Torkar and Vengušt (2008)** analyzed 60 samples of raw milk for the presence of moulds and yeasts, the yeasts were present in 95.0% of raw milk samples with the mean concentration of 1.7 log CFU.mL$^{-1}$. Moulds were found in 63.3% of raw milk samples with mean concentration 0.6 log CFU.mL$^{-1}$.

## CONCLUSION

The microbiology quality of raw and processed milk after storage were evaluated. The results of the present study show that all samples of fresh milk meet the requirements of legislation for number of TVC. However, all samples of raw milk stored for 4 days at 4 °C exceeded the limit for TVC, so raw milk is not suitable for consumption. Milk at direct milk sale points must be stored at 2.5 °C to 4 °C and it must be heat treated, which eliminates the possibility of intensive bacterial growth. Educating of farmers about general hygiene practices, can improve the microbiological quality of milk. It is also very important to ensure of high level of hygiene at the obtaining, processing and storage of raw cow's milk.

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