Oct 31st, 10:45 AM - 12:15 PM

# Determination of aerobic mesophilic bacteria as well as coliforms in a raw milk in the region of Prizren, Suhareka and Rahovec

Hyzer Rizani
*University for Business and Technology*, hyzer.rizani@ubt-uni.net

Shkëlzim Ukaj
*University for Business and Technology*, shkelzim.ukaj@ubt-uni.net

Shkumbim Shala
*University for Business and Technology - UBT*, shkumbim.shala@ubt-uni.net

Magbule Rizani

Naser Kamberi
*University for Business and Technology*, naser.kamberi@ubt-uni.net

*See next page for additional authors*

## Presenter Information

Hyzer Rizani, Shkëlzim Ukaj, Shkumbim Shala, Magbule Rizani, Naser Kamberi, Smajl Rizani, and Duresa Uka

# Determination of aerobic mesophilic bacteria as well as coliforms in a raw milk in the region of Prizren, Suhareka and Rahovec

Hyzer Rizani[1], Shkelzim Ukaj[1], Shkumbin Shala[1,] Magbule Rizani[1], Naser Kamberi[1], Smajl Rizani[1], Duresa Ukaj[1]

[1]Faculty of Food and Technology Sciences, UBT- Higher Education Institution, Lagjja Kalabria p.n., 10000 Prishtina, Republic of Kosovo

*Corresponding author; E-mail:* shkelzim.ukaj@ubt-uni.net

**Abstract**

The microbiological quality of raw milk is a key to the quality production of dairy products. Alternation is a term that describes the change of composition, taste and smell at those points where it is inedible for the consumer. Microbial alternation of milk often involves degradation of proteins, carbohydrates and fats of organisms and their enzymes. Milk and dairy consumption has increased considerably in Kosovo over the last decade, and a large part of local production comes from small-scale distributors across the country. In this research, 50 milk samples were taken at some of the cumulative sites and from dairy farms in three Kosovo municipalities (Prizeren, Suhareka and Rahovec). The microbiological quality of the milk samples is analyzed according to official standards. Further, in raw milk, a number of aerobic mesophilic bacteria and number of coliforms were analyzed. Aerobic mesophilic bacteria in fresh milk, used as raw material, did not show more than $2.0\times10^6$ cfu / ml, whereas coliforms were presented at 4cfu / ml.

Keywords: milk, mesophilic, aerobic, coliform, cfu.

## 1. **Introduction**

Milk is an ideal environment, with a high water content, rich nutritional elements and with an almost neutral Ph (Ph 6,4 – 6,8) that favors the growth of many microorganisms. Microorganisms in milk can be classified in two main groups: pathogens and responsible microorganisms of the demolition where some of them can play a multiple role (ex. Bacillus cereus) [2]. Pathogens microorganisms represent a threat to public health.

Due to their enzymes (ex. proteases, peptidase, lipase, esterase, oxidase, polymerase, β-galaktozidaza), responsible microorganisms of demolition are able to hydrolyzate the milk ingridients, such as protein, fat and lactose appropriately, to gain neccesaryingridients for their growth [1].

Such reactions can cause demolition of milk ,accompained by a change of smell and taste, changes in the quality and view of milk [6]. Microorganisms in milk are mainly spread in dirty environments of farm (ex. faeces, straw chessis and soil. Microorganisms of outer part of the suction can be inserted into canal of udder and may cause mastitis (Makovec & Ruegg, 2003) [9]. In conclusion, we can say that sources of contamination are the inadequacy of milking equipment purity, which then pass in milk [3].

## 2. **Material and Methods**

50 samples were taken in some selling points in Prishtina, Rahovec and Lipjan. Immediately,after the samples were taken, from the delivery place, samples must be put in the freezer compartment, then we make sure to mix the samples to complete homogeneity. Afterward,amount of 30ml of milk is transferred in a sample container. For every sample we have used a test tube. The samples are transferred in 0-4 C temperature, until they were brought to the laboratory.

Examination methods of aerobic mesophilebacteria is based in ISO- 4832-1:2013 [7]. Microbiology of food chain- Horizontal methods of microorganisms counting. First part: counting in 30C through pouring technique and the method of Coliforms ISO 4832:2006. The microbiology of food and animal food products-Horizontal method of coliforms counting - Counting technique [8].

For counting of aerob mesophiles are taken two Petrit's plates. The transfer is made for each by steril pipette from 1 ml of sample. Only critical dilutions are taken for inoculin in the Pjetrit's plate, to develop a colony between 150-300 per plate. For the nutritional area are used plate count approximately 12-15 ml in a temperature of 44-47$^0$C in each plate. The duration from the preparation time of the initial dilution to the pouring of the feeding terrain in plates does not last more than 45 minutes.

The plates were carefully mixed and are left to stand in rigid horizontal positions until they are hardened. the plates are incubated in a temperature of 300C ±10C for 72h ±3h.

The counting of colonies were made as followes, plates with more than 15 colonies and less than 300 colonies were counted.

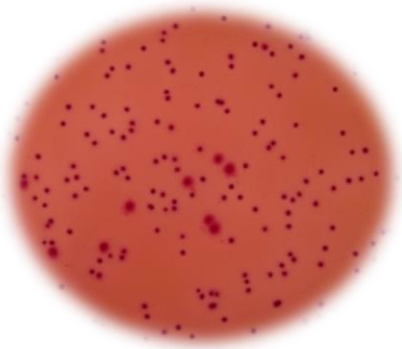

Figure 1: Coliforms in the Violet Bile LactosAgar

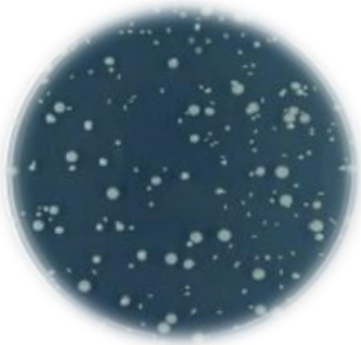

Figure 2: Mesophilic aerobic bacteria on Plate Count Agar

After the incubation, using the calculator is made a counting for specific colonies. For the counting of coliforms is used a hardened nutritional terrain, Crystal violet neutral red bile lactose (VRBL) agar. The microbiology of food and animal food- - Preparation of samples, initial dilution and decimal dilution for microbiological examination. Drying or Moisture Sterilization Equipment (Autoclaves), Incubator, Petri's Plates, 90 mm, Pipettor, 1 ml, Water Baths, counter of colonies,

tube tests, Durham Tubes, lab bottles, pH-meter, Eza from platinum – iridium or nicel-crome, approxiamately 3mm per diameter, or eza for one use.

Two plates are taken, where the tested material was transferred by 1ml steril pipette from the corresponding dillution, in the center of each plate. Then, 15ml of VRBLA are thrown in a temperature of 44 °C - 47 °C in each Petri plate. After the complete hardening, 4 ml of the VRBLA terrain were thrown in 44°C to 47°C, on the surface **o**f inoculated terrains. After the hardening, the plates were inacubated in 37°C per 24 h± 2 h.

After the appointed period of inacubation, Petri's plates with more than 10 colonies and less than 150 colonies were taken for counting. The counting was made using the counting equipment of colonies, the colonist in red with a diameter at least 0/5 mm (sometimes surrounded from the red precipated zones. The confirmation was made by pointing 5 typical colonies, in a liquid area Briliant green lactose, incubated in 37°C temperature.per 24 h± 2 h. There were counted only the colonies that formed gas in Durham tubes. The formula for the counting of the bacterial colonies is as follows:

$$N = \frac{\Sigma C}{V \times (n1 \times 0.1 + n2)} \times d$$

* N – the number of microorganisms in 1 gr sample

*ΣC – the amount of microorganisms in counted plates

*n1 – the number of plates in the intitial dillution

* n2 – the number of plates in the second dillution

*d – the dillution factor.

## 3. **Results and Discussion**

In the table below, are given data microbiological incubators data for every type of fresh milk, for each sample through research proces. The data are the average values of analyzed samples during this study for mesophile bacterias and coliforms in fresh milk. Generally, inside the analyzed type of milk could have been big deviations of measured values.

**Table 1**. The general number of mesophile bacterias and coliforms in fresh milk in the regions of Prishtina, Rahovec and Lipjan.

| Regions | The average of mesophile aerob bacteria | The average number of coliforms |
|---|---|---|
| ***Prizeren*** | *$1.90x10^6$ cfu/ml* | *3.35 cfu/ml* |
| ***Rahovec*** | *$2.60x10^6$ cfu/ml* | *4.06 cfu/ml* |
| ***Suhareka*** | *$2.10x10^6$ cfu/ml* | *4.59 cfu/ml* |
| ***Average*** | *$2.20x10^6$ cfu/ml* | *4.00 cfu/ml* |

From the taken results we can see the total mesophile bacterial microflorais is under the standard norms in three regions: Prizeren1.90x106cfu/ml, Rahovec 2.60x106cfu/ml, Suhareka 2.10x106cfu/ml, and the total average is 2.20x106 cfu/ml. The content of coliform microorganisms is within the limit prescribed by the standard. The high presence of coliform is found in Suhareka region 4:59 cfu / ml, then in Rahovec 4:06 cfu / ml and Prizeren 3:35 cfu / ml.

The concentration of microorganisms depends on the type of microorganisms, the state of infection within the infection, the infestation phase and the infected fecal fraction that constitutes a normal state of milk production and the detection of pathogens mainly transmitted by cows to cows, with or without intermediate mediator. Pollution is mainly transmitted to milk when it is in contact with the outside of the suction and its content decreases during filtration system at milking time [3]. The content of coliform microorganisms is in the described limit from the standard. The highest presence of coliforms is found in the region of Suhareka 4.59 cfu/ml, then in Rahovec 4.06 cfu/ml and in Prizeren 3.35 cfu/ml. Milk contains a variety of microorganisms, however microbial content increases from bedding during fecal contamination. Moreover, the high number of coliforms (7-9log10 cfu g-1) was measured also in used lofts. [10]. In the second year prevails a high load of thermophile bacteria comparing to the content of the bacteria mesophile

## **4. Conclusions**

Bacterial microflora is studied to evaluate the hygiene of production system, before the usage, which then is reflected in the bacterial loads of milk after pasteurization. Every fresh milk tell a total bacterial microflora inside the standard.

During the determination of the number of aerobic mezophilic bacteria in the region of Prizeren we have Starting from the content of coliforms that have resulted in each fresh milk, results in a re-contamination after usage, telling poor hygiene practices. For this reason, it is recommended maintaining of aspectual conditions in the tubes of processing and packaging lines.

In conclusion, we can say that sources of contamination are the inadequacy of the purity of the milking equipment, which then pass on milk.

It is recommended to store the unprocessed milk in $2^0$C, which has resulted to be effective in the growth of fresh milk life comparing to the store beyond $4^0$C and $7^0$C.

## 5. References


1. Andrews, A.T., Anderson, M. &Goodenough, P.W. (1987) A study of the heat stabilities of a number of indigenous milk enzymes. Journal of Dairy Research, 54, 237–246.
2. AMILDA BALLATA "Evaluation of Parameters Increase Products Pasterized (Milk Pasterized) During the Conservation Survey" Dissertation of Scientific Degree DOCTOR.
3. Chambers, J.V. 2002. The microbiology of raw milk. In: Robinson, R.K. (ed.). Dairy microbiology handbook. The microbiology of milk and milk products, 3rd ed. New York, USA: John Wiley and Sons, Inc. pp. 35-90.
4. FAO/WHO (2003c) The Recommended International Code of Practice General Principles of Food Hygiene CAC/RCP 1 –1969, Revision 4 (2003). In: Food HygieneBasic Texts, pp. 1–30, Secretariat of the Codex Alimentarius Commission Joint FAO/WHO Food Standards Programme, Rome.
5. FAO (2003) General Principles of Food Hygiene, Vol. 1, 4th revision of the 1969 edition, pp. 31–32, Codex Alimentarius of the Food and Agriculture Organisation of the United Nation Rome.
6. Frank, J.F. & Hassan, A.N. 2003. Microorganisms associated with milk. In: Roginski, H., Fuquay, J.W. & Fox, P.F. (eds.). Encyclopedia of dairy sciences. London, UK. Academic Press, Elsevier Science. pp. 1786-1796.
7. https://www.iso.org/about-us.html.
8. https://www.iso.org/standard/53728.html.
9. Makovec JA, Ruegg PL. Results of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. J Dairy Sci. 2003 Nov;86(11):3466-72.PubMed PMID: 14672176.
10. Knappstein, K., Roth, N., Walte, H.G. & Reichmuth, J. (2004b) Hygiene Measures Resulting in Adequate Teat Cleaning, Deliverable D15, pp. 4–26, Institute for Hygiene and Food Safety, Federal Research Centre for Nutrition and Food, Kiel, Germany.