

RESEARCH ARTICLE

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Determination of Aerobic Mesophilic Bacteria and Coliforms in Raw Milk in the Region of Kosovo

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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 are taken at some of the cumulative sites and from dairy farms in three Kosovo municipalities (Prishtina, Lipjan 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 2.0×10^6 cfu / ml, whereas coliforms were presented at 4 cfu / 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 present in milk can be classified in two main groups: pathogens and microorganisms that are responsible for the devastation where some of them can play a multiple role (ex. *Bacillus cereus*) [2]. Pathogenic microorganisms represent a threat to public health. Due to their enzymes (ex. protease, peptidase, lipase, esterase, oxidase, polymerase, β -galaktozidaza), responsible microorganisms spoilage are capable to hydrolyzate the milk ingredients, such as protein, fat and lactose, to gain necessary ingredients for their growth [1]. Such reactions can cause milk spoilage, accompanied by a change of smell and taste, changes in the quality and view of milk [6]. Microorganisms in milk are mainly spread from environment of dirty farm (ex. faeces, straw chesis and soil). Microorganisms of outer part of the suction can be inserted into canal of

udder and may cause mastitis [9]. In conclusion, we can say that contamination sources have a lack purity of the milking equipment, which then pass in milk [3].

2. Material and Methods

50 samples are taken in some selling points in Prishtina, Rahovec and Lipjan. Immediately after the samples are taken, from the delivery place, the samples are put in the refrigerator carriers, and then are assured to mix the samples to complete homogeneity. Afterward, amount of 30 ml 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 mesophile bacteria 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 Coliforms method ISO 4832:2006. The microbiology of food and animal food products-

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(Accepted for publication March 20, 2018)

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 with steril pipette from 1 ml of sample. Only critical dilutions are taken for inoculin in the Petrit'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°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 follows, plates with more than 15 colonies and less than 300 colonies were counted.

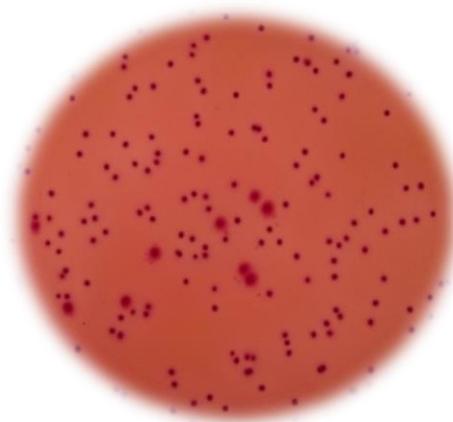


Figure 1: Coliforms in the Violet Bile Lactos Agar

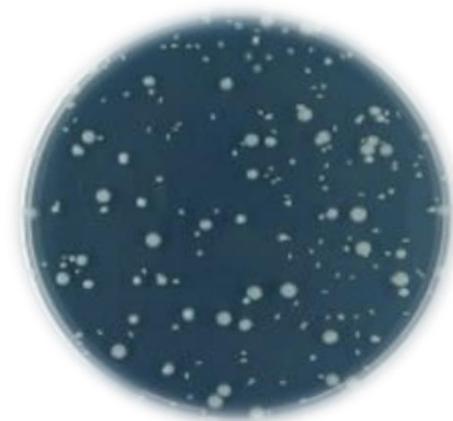


Figure 2: Mesophilic aerobic bacteria on Plate Count Agar

After the incubation, is made a counting for specific colonies by using calculator. 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, Petrit's Plates, 90 mm, Pipettor, 1 ml, Water Baths, colonies counter, tube tests, Durham Tubes, lab bottles, pH-meter, Eza made of platinum – iridium or

nicel-crome, approximately 3mm per diameter, or eza for one use.

Two plates are taken for testing, where the tested material is 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 Petrit's plate. After the hardening completed, 4 ml of the VRBLA terrain are thrown in 44°C to 47°C, on the surface of inoculated terrains. After the hardening, the plates are incubated in 37°C per 24 h± 2 h.

After the appointed period of incubation, Petri's plates with more than 10 colonies and less than 150 colonies are taken for counting. The counting is made by 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 is made by pointing 5 typical colonies, in a liquid area Brilliant green lactose, incubated in 37°C temperature.per 24 h± 2 h. There are 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 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 might have been huge deviations of measured values.

From the taken results we can see the total mesophile bacterial microflora is under the standard norms in three regions: Prishtina 1.90x10⁶cfu/ml, Rahovec 2.60x10⁶cfu/ml, Lipjan 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 Lipjan region 4.59 cfu / ml, then in Rahovec 4.06 cfu / ml and Prishtina 3.35 cfu / ml.

Table 1. The average of general number of the mesophile bacterias and coliforms in fresh milk in the researched regions

Regions	The average of mesophile aerob bacteria	The average number of coliforms
<i>Prishtina</i>	1.90x10 ⁶ cfu/ml	3.35 cfu/ml
<i>Rahovec</i>	2.60x10 ⁶ cfu/ml	4.06 cfu/ml
<i>Lipjan</i>	2.10x10 ⁶ cfu/ml	4.59 cfu/ml
<i>Average</i>	2.20x10 ⁶ cfu/ml	4.00 cfu/ml

The concentration of microorganisms depends on the type of microorganisms, the state of infection within 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 Lipjan 4.59 cfu/ml, then in Rahovec 4.06 cfu/ml and in Prishtina 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 deciduous of milk after pasteurization. All fresh commercial milk represents a bacterial total microflorous within the standard.

Beginning from the content of coliforms that has resulted in all fresh milk ,results in a recontamination after use which indicates poor hygiene practices, it is therefore recommended to maintain aseptic conditions in the processing line tubes and packaging.

It is also recommended that unprocessed milk to be stored in 2⁰C, which has resulted to be effective in the grown of fresh milk life comparing to the store beyond 4⁰C and 7⁰C.

5. References

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