



## COMPARISON OF TOTAL BACTERIAL COUNT (TBC) IN BULK TANK RAW COW'S MILK AND VENDING MACHINE MILK

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### ABSTRACT

Many of raw cow's milk suppliers in Slovakia decided to increase their profit through direct sale of milk from dairy farm or by using milk vending machines. Slovakia has currently about two hundred milk vending machines. Since the machines sold raw milk it is necessary to maintain good milk quality. The objective of this study was to determine bacteriological quality of raw cow's milk sold in vending machines. In this work we also compared total bacterial count in bulk tank raw cow's milk and vending machines milk respectively. From the observation we 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<sup>-1</sup> (5.00 log<sub>10</sub> CFU.ml<sup>-1</sup>) according the European Commission Regulation No. 1662/2006. We have found the average value of total bacterial count in bulk tank raw cow's milk samples 4.61 log<sub>10</sub> CFU.ml<sup>-1</sup> and average value of total bacterial count in vending machine raw cow's milk samples 4.76 log<sub>10</sub> CFU.ml<sup>-1</sup>.

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### 1. Introduction

Milk and milk products provide a wealth of nutrition benefits. But raw milk can harbour dangerous microorganisms that can pose serious health risks. Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, microbial contamination can generally occur from three main sources: from within the udder, from the exterior of the udder, and from the surface of milk handling and storage equipment (Murphy and Boor, 2010). The health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning and sanitizing the milking and storage

equipment are important in influencing the level of microbial contamination of raw milk. Equally important are the temperature and length of time of storage, which allow microbial contaminants to multiply and increase in numbers. All these factors will influence the total bacteria count (Murphy and Boor, 2010). Increased total bacterial count can be caused by growth of bacteria on unsanitary milking equipment, contamination from soiled cow udders, occasionally by milking of mastitic cows. The total bacterial count increases mainly in inadequately cooled milk. The poor hygiene increases the risk of presence of pathogenic bacteria in raw milk (Murphy and Boor, 2000; Hayes et al., 2001; Chambers, 2002; Costello et al., 2003, Elečko et al., 2004,

Zajác et al., 2008, Pantoja et al., 2009, Dudriková, 2010). The presence of pathogenic bacteria in raw milk was studied with several authors (Vasil' et al., 2004, 2008, 2010, 2012, Pyörälä and Taponen, 2009). The resistance of bacteria to sanitising agents and the effectiveness of sanitation process were studied with (Kalmus et al., 2011, Lavová et al., 2010, Čanigová et al., 2004, Fabianová et al., 2010, 2011). Other sources of bacteria contaminating raw milk may be: equipment, hoose, transport containers, equipment of vending machines, personnel and environment (Čapla et al., 2008, Zajác et al., 2011). With the number of bacterial count increase the risk of drug residues in raw milk (Zajác et al., 2004). Soiled udders and teats are common sources of faecal contamination and often indicate inadequate premilking cow preparation. Increased numbers of coliforms in bulk milk can also occur when coliforms grow on residual milk left on milk contact surfaces or in poorly sanitized milking equipment (Guterbock and Blackmer, 1984; McKinnon et al., 1990; Chambers, 2002). Our microbiological survey suggested appropriate preventive measures that should be applied in terms of risk management (Valík et al., 2011). Microbial contamination of raw milk can occur from a variety of microorganisms from a variety of sources. Because of this, determining the cause of bacterial defects is not always straightforward. Although there is often one source of bacteria that cause high bulk tank counts, high bacteria counts can also result from a combination of factors i.e., dirty equipment and marginal cooling (Murphy and Boor, 2010). The problematic of contamination of raw milk was studied Vasil' et al., (2010). Raw milk must be immediately after milking placed in a clean place which is designed and equipped so as to avoid contamination, and cooled to a temperature of not more than 8°C and less than 4°C. In the case of raw milk intended for sale not cooled must be sold within two hours to the final consumer. If this milk is not soled within two hours, it must be immediately cooled to a temperature of up to

+8 ° C and +4°C at least and sold within 24 hours of milking.

Date of consumption of not cooled raw milk intended for direct sale is not more than 24 hours of milking. Date of cooled raw milk is not more than 48 hours of milking. Direct sale of raw milk to the final consumer at the holding milk production takes place in a separate room from the premises where animals are housed and equipped with a cooling device. When producer of milk delivers milk to approved establishments for the collection or processing of milk, then the room for the direct sale of raw milk must be separated from milk rooms. Primary producer in an appropriate manner in a prominent place at the place of sale of raw milk must place these information and data: a) Notice for the end consumer, "Before eating raw milk should be boiled. It is not suitable for direct consumption of children, sick and old people or people with weakened immune" to be stated the type of raw milk; b) The date the consumption of raw milk with the words "use by" and the date and storage conditions of raw milk; c) Information about: name, surname and address of the primary producer or a trade name and place of business, if it is a primary producer who is a natural person - entrepreneur, or a business name and seat of the food business operator, if it is a primary producer, which is a legal entity, breeding for milk production.

Leading primary producer in addition to records relating to traceability and hygiene must have special written records, which shows the total amount of produced raw milk, which has been sold: a) Each day during each buyer; b) During the calendar year.

Primary producer keep records for at least one year after the calendar year in which they were made, and, on request, make available to the competent veterinary authority (Decree of Government of the Slovak Republic no. 360/2012).

Analysis of consumers' opinion on organic food and their safety and availability in the Slovak food market studied Kozelová et al., (2006, 2010, 2011a, 2011b).

From above mentioned, it is necessary to eliminate contamination of raw cow's milk and prevent bacterial growth with storage of milk in proper temperatures. In this work we were compared total bacterial count in bulk tank raw cow's milk and vending machines raw cow's milk. We were focused on the risk of secondary contamination of milk and we have quantified this risk. Milk vending operators should be aware of what may be at risk for the consumer. They should perform a regular sanitation of all equipment that comes into direct contact with the milk. The same attention should be given to the vessels used for the transport of raw milk. The bottles, which are filled in raw milk, must be clean and materials suitable for contact with food. Also, consumers should be aware that raw milk consumption increases the risk of disease caused by the presence of pathogenic bacteria. The all milk vending machines in the Slovak Republic must be in accordance with applicable legislation states that raw milk can be consumed only after cooking.

## 2. Materials and methods

### 2.1. Milk samples

We used samples of raw cow's milk taken from bulk tanks in dairy farms and dairy vending machines located in western area of Slovakia. Bulk tank milk samples were taken directly from dairy farms after the morning milking 6 to 8 a.m. Subsequently, the farmers fill this milk into the vending machines, from which we were sampling second sample. Vending machines milk samples were taken in the afternoon at 12 to 14 p.m. and analysed within the same day at 16 p.m. Milk samples were collected in sterile sample bottles in a volume of 500 ml. Samples were taken in accordance with standard ISO 707:2010. Samples were transported in portable dry ice box at 1 to 5 °C to the National Reference Laboratory for milk and milk products in Nitra, which is accredited according to the international standard ISO 17025 where the samples were subjected to microbial testing. We have analysed total number of 70 bulk tank

raw cow's milk samples and 70 dairy vending machines raw cow's milk samples. Each bulk tank raw cow's milk sample corresponded with vending machines raw cow's milk sample.

### 2.2. Testing period

Samples were collected and analysed during the years 2010, 2011 and 2012.

### 2.3. Total bacterial count analysis

Total bacterial counts in raw cow's milk samples were determined by the standard plate count method, which is recognized as the standard method for enumerating total bacteria count in raw milk. Laboratory testing was performed according to the requirements of these standards: a) STN EN ISO 4833 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 degrees C; b) STN EN ISO 7218 Microbiology of food and animal feeding stuffs. General requirements and guidance for microbiological examinations; c) STN EN ISO 6887-1 Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilutions; d) STN EN ISO 6887-5 Microbiology of food and animal feeding stuffs. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 5: Specific rules for the preparation of milk and milk products.

To determine total bacterial count in raw cow's milk samples OXOID Plate Count Agar (Tryptone Glucose Yeast Agar) code CM0325 was used.

### 2.4. Statistical analysis

We have performed basic statistical analysis in the R language ([www.r-project.org](http://www.r-project.org)).

## 3. Results and discussions

The total bacterial count reflects microbial quality of raw cow's milk. In the Slovak

Republic, the evaluation of the microbiological quality of milk is based on requirements of: Slovak Technical Standard STN 57 0529 : 1999 (Raw cow milk for dairy treatment and processing and European Commission Regulation no. 1662/2006 of 6 November 2006 amending Regulation (EC) No. 853/2004).

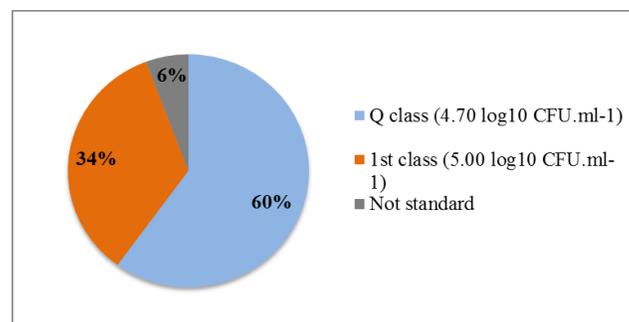
The European Union currently imposes a regulatory limit of  $100.103 \text{ CFU.ml}^{-1}$  ( $5.00 \log_{10} \text{ CFU.ml}^{-1}$ ) of raw cow's milk (EC reg. no. 1662/2006). Slovak Technical Standard STN 570529 determines limit max  $50.103 \text{ CFU.ml}^{-1}$  ( $4.70 \log_{10} \text{ CFU.ml}^{-1}$ ) and  $100 \times 10^3 \text{ CFU.ml}^{-1}$  ( $5.00 \log_{10} \text{ CFU.ml}^{-1}$ ) for Q (quality) and 1st class respectively.

The results of total bacterial count of bulk tank raw cow's milk samples collected from dairy farms in Slovakia show that 60% of samples met Q class limit and 34% met 1st class limit presented in STN 570529. We have found that, 6% of samples were not conforming to legislation limit presented in Regulation (EC) No. 1662/2006 and STN 570529 respectively. These results are presented in the Figure 1.

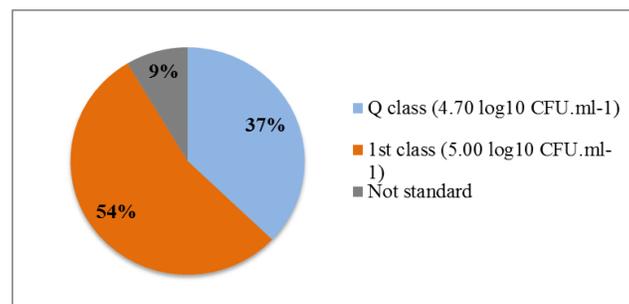
The results of total bacterial count of raw cow's milk samples collected from vending machines in Slovakia show that 37% of samples met Q class limit, 54% met 1st class limit presented in STN 570529. We have found that, 9% of samples were not conforming to legislation limit presented in Regulation (EC) No. 1662/2006 and STN 570529 respectively. These results are presented in the Figure 2. We have found the average value of total bacterial count in bulk tank raw cow's milk samples was  $4.61 \log_{10} \text{ CFU.ml}^{-1}$ . The percentage of unsatisfactory results did not mean that farmers had to be immediately penalised, because in Slovakia a rolling geometric mean is used, according to the Commission Regulation EC No. 1662/2006.

However, there is a large potential to improve the quality and safety of raw cow's milk, as well as economic losses in dairy farms in Slovakia (Zajác et al., 2011). We have found the average value of total bacterial count in

vending machine raw cow's milk samples was  $4.76 \log_{10} \text{ CFU.ml}^{-1}$ .



**Figure 1.** Evaluation of total bacterial count in bulk tank raw cow's milk samples

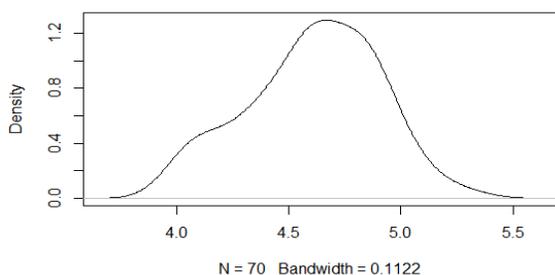


**Figure 2.** Evaluation of total bacterial count in vending machines raw cow's milk samples

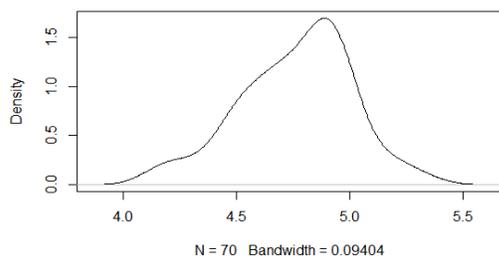
In our previous study we were analysed 19,830 results of total bacterial count of bulk tank raw cow's milk samples taken from dairy farm directly with dairy companies in Slovakia in 2011. These results shown that 84.54% of samples tested for total bacterial count, met the European Union legislation limits and 15.47 % of samples did not met the legislation limit the European Union legislation. The average value of total bacterial count in bulk tanks raw cow's milk samples was  $4.84 \log_{10} \text{ CFU.ml}^{-1}$  (Zajác et al., 2011)

Valík et al., (2011) were analysed microbiological quality of raw cow's milk from vending machines. The analyses and evaluations were focused to bacterial indicators only. Refer to total bacterial counts, the criterion  $\leq 100,000 \text{ CFU.ml}^{-1}$  met 12 from 15 samples (80%) and all samples complied with

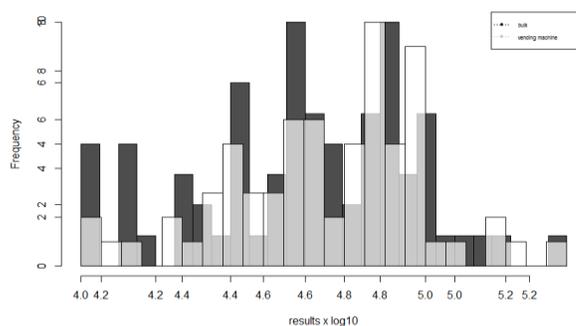
the supplementary criterion of  $5 \times 10^4$  CFU.ml<sup>-1</sup> for psychrotrophs. The average value of total bacterial count in vending machine raw cow's milk was  $4.75 \log$  CFU.ml<sup>-1</sup>. This value is in agreement with our average result of vending machine raw cow's milk total bacterial count  $4.76 \log_{10}$  CFU.ml<sup>-1</sup>. Assuming a constant rate of bacterial growth we may expect the increase in the numbers of different groups of microorganisms after 24 hours and we can also assess the density quite well after 48 hours. The average total bacterial count value after 24 h increased by one logarithmic order of  $5.8 \log_{10}$  CFU.ml<sup>-1</sup>. Based on this assumption, the number of bacteria after 48 h should achieve an average of  $6.8 \log_{10}$  CFU.ml<sup>-1</sup> ( $6.3 \cdot 10^6$  CFU.ml<sup>-1</sup>) (Valík et al., 2011). Histogram of total bacterial count in bulk tank raw cow's milk samples is presented in Figure 3. Histogram of total bacterial count in vending machines raw cow's milk samples is presented in Figure 4. Histogram of paired t-test of total bacterial count in bulk tank raw cow's milk samples and vending machines raw cow's milk samples. We were confirmed our hypothesis (p-value <0.001), that we can expect increase the number of bacteria in the raw cow's milk in vending machine.



**Figure 3.** Histogram of total bacterial count in bulk tank raw cow's milk samples



**Figure 4.** Histogram of total bacterial count in vending machines raw cow's milk samples



**Figure 5.** Histogram of total bacterial count in bulk tank raw cow's milk samples and vending machines raw cow's milk samples

#### 4. Conclusions

In this research, we focused on the comparison of microbial quality of raw cow's milk from bulk tank and vending machines.

The results of total bacterial count of bulk tank raw cow's milk samples collected from dairy farms in Slovakia show that 60% of samples met Q class limit and 34% met 1st class limit presented in Slovak technical standard STN 570529. We have found that, 6% of samples were not conforming to legislation limit presented in Regulation (EC) No. 1662/2006 and STN 570529 respectively. The results of total bacterial count of raw cow's milk samples collected from vending machines in Slovakia show that 37% of samples met Q class limit, 54% met 1st class limit presented in Slovak technical standard STN 570529. We have found that, 9% of samples were not conforming to legislation limit presented in Regulation (EC) No. 1662/2006 and Slovak technical standard STN 570529 respectively. We have found the average value of total bacterial count in bulk tank raw cow's milk samples  $4.61 \log_{10}$  CFU.ml<sup>-1</sup> and average value of total bacterial count in vending machine raw cow's milk samples  $4.76 \log_{10}$  CFU.ml<sup>-1</sup>. Comparing the results of total bacterial count of bulk tank milk samples and milk samples obtained from vending machines we verified that presence of secondary

contamination of milk during the transport and storage. Also, we can expect intensively bacterial growth. Despite the fact that most of raw cow's milk (94% bulk tank and 91% vending machines) samples meet hygiene limits for total bacterial count, it is necessary to perform heat treatment of this milk before the consumption, to reduce the risk of foodborne illness.

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