Microbiological status of bulk tank milk and different flavored gomolya cheeses produced by a milk producing and processing plant

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SUMMARY

The microbiological quality of milk is important not only for food safety, but it can also influence the quality of dairy products. In this study, our aim was to assess the microbiological status of the bulk milk of a milk-producing farm, and some natural and flavored (garlic, dill, onion) gomolya cheeses made from pasteurized milk produced by their own processing plant. We determined the number of coliform bacteria, Escherichia coli, Staphylococcus aureus, and molds of three milk and eight cheese samples. The tests were conducted between July and September, 2017.

In bulk milk, the mean coliform count was $3.83 \pm 0.17 \log_{10}$ CFU/ml; the mean E. coli count was $1.38 \pm 0.14 \log_{10}$ CFU/ml; the mean mold count was $3.74 \pm 1.30 \log_{10}$ CFU/ml; and the S. aureus count was $<1.00 \log_{10}$ CFU/ml, respectively. The mean coliform count in gomolya cheeses was $3.69 \pm 1.00 \log_{10}$ CFU/g; the mean E. coli count was $2.63 \pm 0.58 \log_{10}$ CFU/g; the mean S. aureus count was $3.69 \pm 1.35 \log_{10}$ CFU/g and the mean mold count was $1.74 \pm 0.37 \log_{10}$ CFU/g. The amount of coliforms detected in different flavored gomolya cheeses were significantly different (P<0.05). More than 10 CFU/g of E. coli was found only in the dill flavored cheeses, and S. aureus was found only in dill ($3.66 \pm 1.86 \log_{10}$ CFU/g) and onion ($3.71 \pm 0.52 \log_{10}$ CFU/g) flavored gomolya cheeses. Based on the obtained results, it was found that the amount of coliform bacteria and E. coli in bulk milk exceeded the limit set in regulation of the Hungarian Ministry of Health (MoH) 4/1998 (XI. 11.) and the amount of S. aureus was below the limit. For gomolya cheeses, the S. aureus count exceeded the limit. The amount of coliform bacteria remained above the limit in cheeses, except for the garlic flavored gomolya cheese. In cheeses, a larger E. coli count was detected than in the bulk milk, but there is no specific limit for cheeses in the regulation. The mold count exceeded the limit specified in the regulation in cheeses, but a lower value was detected relative to milk.

The results show that, in the case of bulk milk and gomolya cheeses, certain detected quantities exceeded the limit values set forth in regulation of MoH 4/1998 (XI. 11.). The results indicate an inadequate microbiological state of the raw material and the finished products. The reasons for these are due to reduced technological hygiene or the inappropriate handling of raw material and finished products. In this study, we have summarized the results of our preliminary studies, which can provide a basis for further hygiene studies.

Keywords: microbiology, bulk milk, gomolya cheese

INTRODUCTION

The milk contains some nutrients which are important for the human body, for example proteins, fats, vitamins, minerals and water. These nutrients are also needed for microbes; therefore, many microorganisms can be present in the milk, including pathogenic organisms, e.g. *Staphylococcus aureus* (Akindolire et al. 2015). In addition, the high water activity and neutral pH of the milk provide optimum conditions to their growth (Deák 2006, Quigley et al. 2011).

The milk of a healthy cow contains a small amount of microbes, it is considered sterile in the udder, but during handling of milk after milking it can easily be contaminated (Biró 2014). Contamination may occur for example from the skin of the animals, from the environment or from milking machines, milk lines and storage tanks. Mostly, heat treatment is used to reduce the number of bacteria. The initial microbiological quality of the milk is important not only for food safety, but it can also influence the quality of the dairy products (Cilliers et al. 2014).

Some of the most popular dairy products are cheeses, which have many varieties and forms around

the world (El-Hofi et al. 2010). The cheese making process starts with the curdling of milk, followed by the molding of curd. The cheese is then pressed, salted, and then, for certain types of cheeses, maturation follows (Laczay 2008). The Codex Alimentarius Hungaricus Directive Number 2-51 (2004) contains requirements for milk and dairy products. According to this directive, the gomolya cheeses are made with mixed (acidic and enzymatic) curdling and can be consumed immediately after production, so gomolya cheeses can be regarded as fresh cheese.

According to the Codex Alimentarius Hungaricus, for the manufacture of dairy products the materials should meet the relevant requirements, national recommendations, commercial requirements and dietary goals.

Food manufacturing companies try to meet consumers' expectation of having safe and high quality products on their desk by operating rigorous quality management systems. However, despite their efforts, there may be problems with the presence of microbes (e.g. *S. aureus*, *Escherichia coli*, etc.) (El-Hofi et al. 2010). The contamination of dairy products may occur from the dairy plant itself or from the farms due to improper hygiene practices (Campolo et al.

2013). Hygiene indicator microorganisms can represent a picture of the microbiological status of foodstuffs and their environment. Indicator microbes are for example coliform bacteria, *E. coli* and molds (Vasek et al. 2008, Campolo et al. 2013, Martin et al. 2016).

For about a century, the coliform bacteria have been used as indicator microorganisms. These bacteria are gram-negative, have aerobic or facultative anaerobic properties and do not produce spores. They can ferment lactose at 32–35 °C, while they produce acid and gas (Martin et al. 2016). As they are generally present in the environment, their presence in food may indicate environmental contamination. The coliform bacteria include *E. coli*, which is considered to be a frequent pollutant of raw and processed milk. *E. coli* is found in the intestinal tract of most mammalian species so it can be a fresh faecal contamination indicator. It can get into different foods (like milk and dairy products) from different sources. The presence of *E. coli* may indicate the presence of enteropathogenic and/or toxigenic microbes (Altalhi and Hassan 2009, Mhone et al. 2011).

Milk provides excellent conditions for the growth of *Staphylococci* and their production of enterotoxin. Enterotoxins produced by enterotoxin-producing *S. aureus* strains can cause food poisoning in people who consume food contaminated with this bacterium. The symptoms (diarrhea, vomiting, abdominal cramps) appear 1 to 8 hours after the contaminated food is consumed. In dairy farms, raw milk may be contaminated with the bacteria from the environment, from the hands of the milkers, from the milking equipment and from the animal skin (Korpysa-Dzirba and Osek 2011).

Molds are often found in raw milk but don't survive pasteurisation. In pasteurized milk or dairy products, they may occur when re-infection happens during manufacturing. In raw milk some molds play a role in manufacturing dairy products (e.g. *Penicillium camemberti*, *Penicillium roqueforti*), but the presence of some type of molds is undesirable, because they can impair the organoleptic properties of the dairy products or even pose a health hazard through the production of mycotoxins (Wouters et al. 2002, Torkar and Teger 2006). Molds can also be considered as indicators of environmental contamination (Vasek et al. 2008).

In this study our aim was to assess the microbiological status of the bulk milk of a milk-producing farm, and some nature and flavored (garlic, dill, onion) gomolya cheeses made from pasteurized milk produced by their own processing plant. We determined the number of some indicator microbes, i.e. coliform bacteria, *E. coli* and molds, and we also determined the amount of *S. aureus*. As a conclusion, we suggest further hygiene studies to gain a better understanding of the microbiological status of the dairy products.

MATERIALS AND METHODS

Place and date of sampling

For the microbiological examination we collected bovine bulk milk samples (n=3) in sterile plastic sample tubes from a milk producing medium-sized farm. The housing technology used in the farm is deep litter. The milking of the Hungarian spotted cattle breed is done in milking parlour.

The milk is processed in the farm's own dairy plant, and a variety of dairy products, for example different flavored gomolya cheeses are produced, and then sold in the farm's own retail units. In this study, 8 gomolya cheeses in 4 flavors (natural, garlic, dill, onion) were examined. The samples were tested at the Microbiological Laboratory of the Institute of Food Science, University of Debrecen (Hungary). The tests were carried out in July, August and September of 2017.

Microbiological analysis

Preparations for the tests were carried out in accordance with the MSZ EN ISO 6887-1 (2000) standard. The milk sample was stored in the refrigerator (on 4 °C) until testing, and homogenized by shaking before the decimal dilutions were prepared. For preparation of cheese samples, cheese packaging was removed and then 10 grams of samples were added to the sterile Stomacher® Bag (Seward Ltd., UK) containing the appropriate sample identification mark. 90 ml of sterile peptone water was added to the sample, in sterile conditions. For one liter peptone water, 8.5 g of sodium chloride (VWR International Ltd., Hungary) and 1.0 g of peptone (Merck Kft., Hungary) were dissolved in distilled water, then sterilized. The sample then was homogenized in lab blender for 2 minutes (paddle speed: 240/min; fixed speed: 8 strokes/s).

To prepare the decimal dilution line, 9 ml of the peptone water was measured in test tubes, which were then sterilized in pressure cooker for 30 minutes at about 120 °C.

Microbiological tests were performed according to standards for the microbes.

The determination of the coliform count was carried out in accordance with the ISO 4832 (2006) standard. Following this, sterile Violet Red Bile Lactose (VRBL) agar (Biolab Ltd., Hungary) was used and the determination was done by pour plate technique. When preforming the pour plate technique, 1 ml from the dilutions was pipetted into sterile plastic Petri dishes, then we poured the medium on it, mixed, allowed to solidify and then put it into the thermostat at 37 °C for 24 hours.

The amount of *E. coli* was determined according to the MSZ ISO 16649-2 (2005) standard. Sterile Tryptone Bile X-Glucuronide (TBX) agar (Biolab Ltd., Hungary) was used, and the samples were prepared by pour plate technique. Incubation lasted for 18 to 24 hours at 37 °C.

The *S. aureus* count was determined by spread plate method, in accordance with the MSZ EN ISO 6888-1 (2008) standard. Baird-Parker agar (Biolab Ltd., Hungary) supplemented with egg yolk tellurit emulsion (LAB-KA Ltd., Hungary) was used, and the plates were incubated at 37 °C for 48 hours. When performing the spread plate technique, 0.1 ml of the dilutions was pipetted onto the medium and then spread by a sterile glass rod. The identification of *S. aureus* was performed by latex agglutination test kit (Prolex Staph Xtra Kit, Ferol Ltd., Hungary). The evaluation of the amount of *S. aureus* in bulk milk was performed according to the regulation of the Hungarian Ministry of Agriculture and Regional Development and the Hungarian Ministry of Health, Social and Family Affairs 1/2003 (I. 8).

The determination of the mold count was carried out in accordance with the MSZ ISO 21527-1 (2013) standard. Dichloran Rose Bengal Chloramphenicol (DRBC) agar (VWR International Ltd., Hungary) was used, and the determination was done by spread plate technique. The plates were incubated at 25 °C. Because the rapidly growing molds can be problem, the colonies were counted after 2 days, and then again after 5 days.

Statistical analysis

Calculation of averages, standard deviations (SD), logarithmic transformation of the amount of microorganisms, t-tests and variance analysis were performed using the SPSS v.22.0 (SPSS 2013) software.

RESULTS AND DISCUSSION

The mean coliform count in bulk milk was 3.83 ± 0.17 $\log_{10}$ CFU/ml, so it was a higher colony count than the limit (m=1.00 $\log_{10}$ CFU/ml) set in the regulation of Hungarian Ministry of Health 4/1998 (XI.11). In the case of cheeses, lower colony count than 2.00 $\log_{10}$ CFU/g was measured in the garlic flavored gomolya cheeses, so that's why it is not shown in *Figure 1*.

Figure 1: **Coliform count in bulk milk and in gomolya cheese samples**
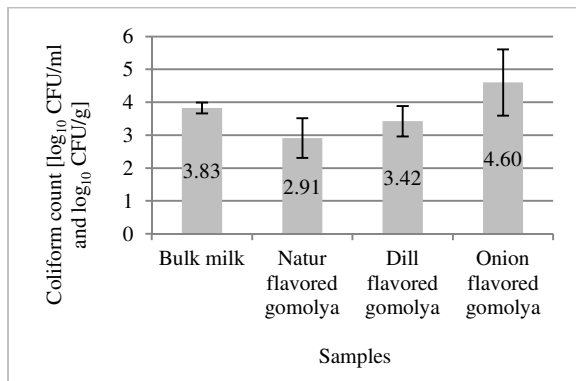
In case of the other flavors, higher values were detected than the limit (m=1.00 $\log_{10}$ CFU/g). In the natural flavored cheeses the mean coliform count was 2.91 ± 0.61 $\log_{10}$ CFU/g, in the dill flavored cheeses it was 3.42 ± 0.46 $\log_{10}$ CFU/g, and in the onion flavored cheeses it was 4.60 ± 1.00 $\log_{10}$ CFU/g. The highest value was detected in the onion flavored gomolya, and the results of them are significantly different from the results of the other cheeses (P<0.05).

In the bulk milk samples, the mean *E. coli* count was 1.38 ± 0.14 $\log_{10}$ CFU/ml, so the limit (m=0.00 $\log_{10}$ CFU/ml) was exceeded. In the gomolya cheeses, less than 1.00 $\log_{10}$ CFU/g of *E. coli* was present, except for the dill flavored cheeses, so the results of the other gomolya cheeses couldn't be illustrated in *Figure 2*. The amount of *E. coli* in dill flavored gomolya cheeses (2.63 ± 0.58 $\log_{10}$ CFU/g in average) was higher than in the bulk milk, but the difference was not significant (P>0.05). The limit for *E. coli* in cheeses is not specified in the regulation.

Figure 2: **E. coli count in bulk milk and in dill flavored gomolya cheese samples**
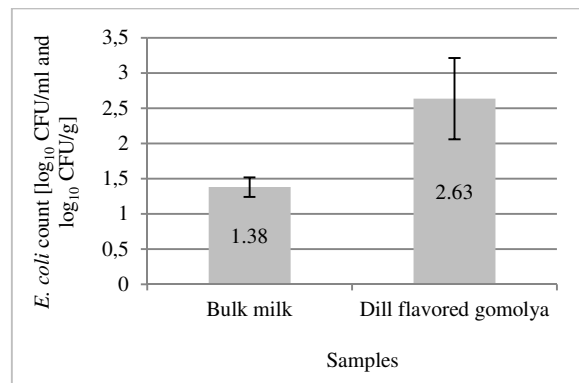
Less than 1.00 $\log_{10}$ CFU/ml of *S. aureus* was detected in bulk milk, so it was less than the limit (m=2.70 $\log_{10}$ CFU/ml) set in the regulation of the Hungarian Ministry of Agriculture and Regional Development and the Hungarian Ministry of Health, Social and Family Affairs 1/2003 (I. 8). In the natural and garlic flavored gomolya cheeses less than 1.00 and 3.00 $\log_{10}$ CFU/g *S. aureus* were detected, hence these results are not shown in *Figure 3*. In the dill and onion flavored gomolya cheeses the mean *S. aureus* count was 3.66 ± 1.86 $\log_{10}$ CFU/g and 3.71 ± 0.52 $\log_{10}$ CFU/g. There was no significant difference between these results (P>0.05). The limit for *S. aureus* in cheeses is 0. The contamination of the cheeses with the bacterium can occur during manufacturing handling of the finished products.

In bulk milk, the mean mold count was 3.74 ± 1.30 $\log_{10}$ CFU/ml, but there is no limit in the regulation related to this. In the onion flavored gomolya cheeses the mold count (1.74 ± 0.37 $\log_{10}$ CFU/g in average) exceeded the limit (m=1.00 $\log_{10}$ CFU/g). There were fewer molds in the finished product, but the difference was not significant (P>0.05). In the further gomolya cheese samples less than 1.00 and 3.00 $\log_{10}$ CFU/g

molds were detected, so these results are not presented in *Figure 4*.

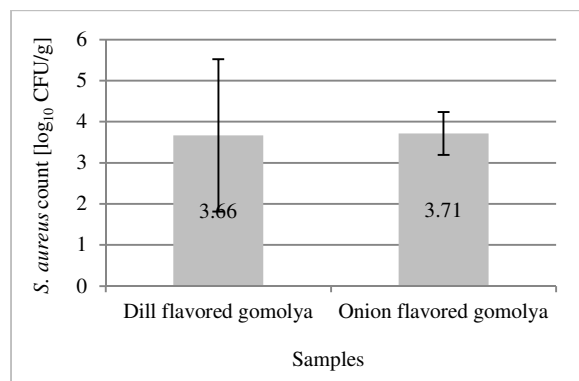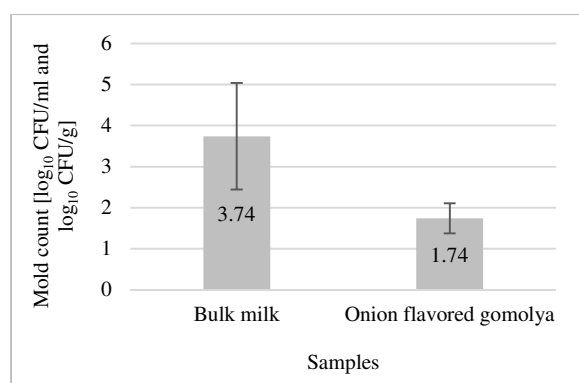*Figure 3*: **S. aureus count in dill and onion flavored gomolya cheese samples**

Figure 4: **Mold count in bulk milk and in onion flavored gomolya cheese samples**

The microbiological quality of the tested bulk milk and gomolya cheese samples is summarized in *Table 1*. In bulk milk samples, the mean coliform count was 3.83±0.17 $\log_{10}$ CFU/ml. Peles et al. (2008) also studied the amount of coliform bacteria in bulk milk

of dairy farms. They found that the mean coliform count in bulk milk of a medium-sized farm was 1.77±1.18 $\log_{10}$ CFU/ml, which was a lower value than the values we detected. El-Hamdani et al. (2016), in their study of bovine raw milk, reported coliform counts of 2.78 $\log_{10}$ CFU/ml and 3.48 $\log_{10}$ CFU/ml for autumn and spring, which were also lower values than the values we detected. In our study, the mean *E. coli* count was 1.38±0.14 $\log_{10}$ CFU/ml, so it was a higher colony count than the mean *E. coli* count (1.09±1.05 $\log_{10}$ CFU/ml) in the study of Peles et al. (2008), and it was a lower mean colony count than in raw milk samples (6.2±5.5 $\log_{10}$ CFU/ml) of smallholder dairy farms examined by Mhone et al. (2011). The mean mold count in this study was 3.74±1.30 $\log_{10}$ CFU/ml, so we got higher mean mold count than Peles et al. (2008), because the mean colony count they detected was 1.03±0.67 $\log_{10}$ CFU/ml. In this study, the amount of *S. aureus* was less than 1.00 $\log_{10}$ CFU/ml in bulk milk. Peles et al. (2007) also studied the amount of *S. aureus* in bulk milk of two farms and obtained 3.15 $\log_{10}$ CFU/ml and 2.41 $\log_{10}$ CFU/ml of *S. aureus*, respectively. So these colony counts were higher than the *S. aureus* count obtained in our study. Mhone et al. (2011) also obtained higher mean *S. aureus* count (5.4±5.1 $\log_{10}$ CFU/ml) when examining raw milk of smallholder dairy farms.

In cheese samples the mean coliform count was 3.69±1.00 $\log_{10}$ CFU/g. The mean *E. coli* count was 2.63±0.58 $\log_{10}$ CFU/g, so it was a lower result than the mean *E. coli* count (6.15 $\log_{10}$ CFU/g) reported by Torkar and Teger (2006), who evaluated the microbiological quality of cheese samples produced at small dairy-processing plants. In our study, the mean *S. aureus* count was 3.69±1.35 $\log_{10}$ CFU/g and the mean mold count was 1.74 ± 0.37 $\log_{10}$ CFU/g, as shown in the *Table 1*. In both cases, lower values were obtained than the colony counts reported by Torkar and Teger (2006), as in their study, the mean S. aureus count was 4.40 $\log_{10}$ CFU/g, and the mean mold count was 4.30 $\log_{10}$ CFU/g, respectively.

Table 1

Microbiological quality of bulk milk and gomolya cheese samples

Sample	Microorganism	Mean±SD [$\log_{10}$ CFU/ml; $\log_{10}$ CFU/g]	Minimum [$\log_{10}$ CFU/ml; $\log_{10}$ CFU/g]	Maximum [$\log_{10}$ CFU/ml; $\log_{10}$ CFU/g]
Bulk milk	Coliform bacteria	3.83±0.17	3.64	3.95
	Escherichia coli	1.38±0.14	1.28	1.48
	Staphylococcus aureus	<1.00	<1.00	<1.00
	Mold	3.74±1.30	2.28	4.78
Cheese	Coliform bacteria	3.69±1.00	<2.00	5.36
	Escherichia coli	2.63±0.58	<1.00	3.30
	Staphylococcus aureus	3.69±1.35	<1.00	5.08
	Mold	1.74±0.37	<1.00	2.00

Note: SD – standard deviation

CONCLUSION

The coliform bacteria can be used as hygiene indicator microbes. As they are generally present in the environment, their presence in the food may indicate environmental contamination (Altalhi and Hassan 2009, Mhone et al. 2011, Martin et al. 2016). Since the hygiene status of dairy products is indicated by the presence of coliform bacteria (including *E. coli*), the amount of them was determined in the bulk milk and gomolya cheese samples. According to our results, in the majority of samples the coliform bacteria were detected above the limit, which is 1.00 $\log_{10}$ CFU/g in the case of cheeses and also 1.00 $\log_{10}$ CFU/ml in bulk milk. So it can be said that the samples were contaminated from the environment, either during processing of the milk or handling the dairy products.

E. coli is often used as a hygiene indicator microbe also, as it can signal direct or indirect faecal contamination, because of its origin (human and animal intestinal tract) (Ombarak et al. 2016). Based on our results, *E. coli* was present in excess of the limit value in the bulk milk, suggesting inadequate hygiene conditions for milk production.

S. aureus can cause significant problems in dairy farms, as one of the microbes responsible for mastitis in dairy animals. They can get into the milk from the animal suffering from mastitis; therefore, the contaminated milk may pose a public health hazard to the consumer. If enterotoxin-producing strains are present in the milk, and the amount of bacteria exceeds 10^5 CFU/ml, food poisoning can occur (Hill et al. 2012). In our studies, less than 10 CFU/ml *S. aureus* was detected in bulk milk, which is below the amount of microbes that would cause a food-borne disease to the consumer. The low amount of colonies indicates that the milk is not contaminated with *S. aureus* either from the animal or the environment. In the dill and onion flavored gomolya cheeses the *S. aureus* count was above the limit. The contamination of the cheeses with the bacterium can occur during making these cheeses or when handling finished products, or the contamination may be got into the products through the spices used as flavoring.

Raw milk or pasteurized milk is used for cheese production. Pasteurization reduces the amount of microbes in milk, including molds. This means that molds can get into the finished products during the production process or when handling the products (Valkaj et al. 2013). In this study, a large amount of mold was detected in bulk milk, which may have several reasons, e.g. their number did not decrease during pasteurization or the milk handling was not adequate after pasteurization.

ACKNOWLEDGEMENTS

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