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RAW MILK HYGIENE AND QUALITY AS AN IMPORTANT PARAMETER FOR OBTAINING QUALITY DAIRY PRODUCTS

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Abstract
The aim of the research was to evaluate the impact of the raw milk's quality and hygiene on dairy products manufactured on a traditional way of production without milk pasteurization and without addition of any starter culture. The chemical and microbiological parameters of the raw milk on the 1st day and cheese samples on the 1st, 5th, 10th, 15th, 30th, 45th and 60th day were obtained. It was observed a negative correlation between content of NaCl and the total bacterial count during the ripening time until the 45th day, but unexpected increasing of the bacteria number was noted on the 60th ripening day. On the 60th day the number of *E. coli* from $3,1*10^2$ increased to $3,4*10^2$ and the total *coliform bacteria* number from $3,8*10^3$ increased to $9,0*10^3$. The number of *Enterobacteriaceae* and *Staphylococcus spp.* also increased from $1,9*10^3$ to $4,7*10^4$ and from $21,5*10^5$ to $29,7*10^5$, respectively. The overall sensorial characteristics of the cheese have shown unsatisfying results due to color, odor and structure.
Key words: cheese, bacterial count, E. coli, S. aureus

Introduction
According to the regulations for quality of the milk and dairy products (Official Gazette no. 96/2011) raw milk is milk obtained as a product of the milk glands of the farm animals and which is not thermal threatened on temperature level higher than 40°C and in which nothing is added or taken. The quality and hygiene of the raw milk are the basic and most essential parameters for producing safe and quality dairy products, especially when they are produced in a traditional way. It all begins with the quality on the farm level. Milk is a good growth medium for many microorganisms because of its high water activity, near neutral pH, and available nutrients (Frank, 2007). The microbiological quality of raw milk can be affected by several factors, such as milking, housing, farming system (organic, conventional), and the season of the year (Bogdanovicova et al. 2016). It is evident that the rate of survival and/or growth of pathogenic bacteria in cheeses depends on the ecological conditions (Aw (water activity), pH, salt content, temperature of

maturation) within the cheese and/or brine (Kostovska et al. 2017). Although storage in brine is thought to cause a decrease in the populations of undesirable contaminants, there is great concern that the brine can also serve as a reservoir of certain salt-tolerant pathogens (Bintsis, and Papademas, 2002). Raw milk quality measurements most often considered in regard to potential effect on processed product quality are the SCC and total bacterial counts. At higher levels, somatic cells and bacteria are associated with increased activity of enzymes that damage milk components and potentially result in product defects (Murphy et al. 2016). According to Kalevska, (2009) manufacturing milk which has lower hygiene quality and in which the number of somatic cells is high causes huge economic loss in the dairy industry because of underuse of the raw material and reducing the final products quality, the cheese yield, appearance of atypical sensorial characteristics and shortening the shelf-life of the product. The presence of bacteria from the family *Enterobacteriaceae* indicates the potential faecal contamination during milking (Ibtisam et al. 2007). *E. coli* is commonly found in the intestinal microflora of humans and warm-blooded animals, but it may become a pathogenic organism (Costa et al. 2009). The presence of *Staphylococcus spp*. such as *Staphylococcus aureus* in raw milk generally comes from cows with mastitis, from handlers or from deficient hygiene. When found in milk, high levels of contamination can be reached quickly under favorable conditions. Its presence in foods can be a risk to human health, causing a public health problem, as these bacteria produces toxins that can cause toxic food infections (de Oliveira et al, 2011).

Material and methods

As a material for this paper was used raw cow's milk. The final dairy product, which was produced with the raw milk was white brined cheese, and it was produced on a traditional way, without milk pasteurization and without addition of any starter culture. After the addition of the liquid rennet, it was noted belated curdling of the milk. For the physico-chemical analysis of the milk was used Lactoscan MCC. Milk sample was examined by Beta star screening kit (Neogen, USA) which is used for rapid detection of the betalactam antibiotics such as penicillin, ampicillin, amoxicillin, cloxacillin, and cephapirin. The pH of milk and cheese samples was measured using a digital pH meter (model MP120FK Mettler Toledo, Greifensee, Switzerland). Titratable activity (TA) was determined by titration using Soxlet-Henkel method. Dry matter and water content was determined by MJ33 Mettler Toledo. The NaCl contents in curd and cheese during ripening were determined by the Mohr method. The determination of the content of milk fat in the milk sample and in the cheese was determined by the Gerber method.

Samples for microbiological analysis were taken under sterile conditions, placed into plastic sterile dishes and kept under refrigeration until analysis. Microbiological analyses were preformed within 2h after the sampling at Anima Vet laboratory (Bitola). The enumeration of *E. coli* was performed according to the

method defined by MKC EN ISO 16649-2:2008, known as Horizontal method for the determination of β-glucuronidase-positive Escherichia coli, by the technique of counting colonies cultured at 44°C, using 5-bromo-4-chloro-3 indolyl-β-d-glucuronide.
The enumeration of *Enterobacteriaceae* was performed according to the method defined by ISO 21528 - 2:2004, known as Horizontal methods for the detection and enumeration of *Enterobacteriaceae*, and the enumeration of *Staphylococcus spp.* was performed according to the method defined by MKC EN ISO 6888 - 2/A1: 2008, known as Horizontal method for the enumeration of *coagulase-positive staphylococc*i, (*Staphylococcus aureus* and other species).

Results and discussion

The quality of the raw cow's milk is presented in Table 1. The obtained results show that the average somatic cell count in the milk was 0,98 $*10^6$ cells/ml and total bacterial count was 5,836$*10^6$ cells/ml which is much higher than allowed regulations (Official Gazette no. 197/2016). This confirms the poor hygienic conditions at farm level. The chemical parameters in milk are in compliance with the requirements (Official Gazette no. 96/2011) also the results obtained in this study confirmed earlier findings of Mojsova et al. (2013).

Table 1: Quality of the raw cow's milk (Kostovka et al. 2017)

Chemical parameters of the milk		**Microbiological parameters of the milk (CFU/ml)**	
Fat (%)	3,80	*Enterobacteriaceae*	0,033 $*10^6$
Solids non fat SNF (%)	8,30	*Coliform bacteria*	5,1 $*10^6$
Density (%)	27,99	*Staphylococcus*	0,42 $*10^6$
Lactose (%)	4,55	*Escherichia coli*	0,0024 $*10^6$
Solids (%)	0.68	Total bacterial count	5,836 $*10^6$
Protein (%)	3,04		
^{0}SH (%)	7,2		
Conductivity (%)	3,43		
Freezing point	-0,528		
Antibiotic residues	No positive detection		
SCC (cells/ml)	0,98 $*10^6$		

In Table 2 and Table 3 are presented the obtained physico–chemical and microbiological results during the 60 days ripening period of the cheese. The

manufactured white brined cheese has shown unsatisfying results due to overall sensorial characteristics such as the structure and the odor which was very intense. According to Auldist et al., (1996) the changes that occur in the milk with huge number of somatic cells have negative effect on its convenience for cheese manufacturing and it is a result of the negative impact of the enzymes on the milk proteins and lipids. According to Levkov et al. (2014) pH should be decreasing as a result of lactic acid production by lactic acid bacteria (LAB), but in our study pH values gradually increased during the ripening period. This might be a result of yeast metabolic activity which uses lactic acid as a source of carbon, or a result of great amounts of alkaline compounds released during proteolytic activities (Volken de Souza et al., 2003). A high incidence of contamination of brine-salted cheeses by yeasts results from their presence in the brines (Kaminarides and Lakos, 1992). Decreasing values of the titratable acidity were noted during the ripening period which might be a result of lack of lactic acid which leads to reduction of titratable acidity and adulteration of cheese during the ripening time (Velevski, 2015). The traditional cheeses contain original microflora (Beresford et al. 2001) which evolved during ripening as a result of nutritive and environmental changes in cheese (Williams et al. 2002). The negative correlation between content of NaCl and the total bacterial count was observed during the ripening time, but unexpected increasing of the bacteria number was noted on the 60^{th} ripening day. The appearance of mold on the cheese surface possibly affected the pH increase which cause bacterial growth in number. According to Frank, (2001) in soft, mold-ripened cheeses, the pH increases during ripening, this increases the growth potential of coliform bacteria. Growth inhibition of *S. aureus* by lactic acid produced from starter culture was suggested to be the cause of growth inhibition in the natural cheese (Aoyama et al. 2008). In our case lack of lactic acid caused an increase of *Staphylococcus spp.*

Table 2: Changes in the physicochemical parameters during manufacturing and ripening of white brined cheese

Parameters	Cheese making		Ripening cheese (days)						
	Milk	Curd	1	5	10	15	30	45	60
pH	6,67	5,30	5,29	5,29	5,47	5,59	5,6	5,75	6,11
Titratable acidity (^{0}SH)	7,2	72	80,8	42	42,4	48,8	32,8	33,6	34,8
Dry matter (%)	12,08	48,94	49,29	48,82	47,02	48	48	48,76	54,34
Water content (%)	/	51.06	50,71	51,18	52,98	52	52	51,24	45,66
Fat (%)	3,9	27	27	27	27	27	27	27	27
NaCl (%)	/	/	1,60	7,68	8,17	8,14	8,14	8,14	9,6

Table 3: Changes in microbiological counts during manufacturing and ripening of white brined cheese

Parameters	Ripening cheese (days)						
	1	5	10	15	30	45	60
Total coliform bacteria (cfu/ml*/cfu/g**)	$13,6*10^6$	$1,7*10^6$	$8,0*10^5$	$9,0*10^4$	$4,5*10^3$	$3,8*10^3$	$9,0*10^3$
Escherichia coli (cfu/ml*/cfu/g**)	$2*10^5$	$1,1*10^5$	$1,1*10^4$	$2,0*10^3$	$5,5*10^2$	$3,1*10^2$	$3,4*10^2$
Enterobacteriaceae (cfu/ml/cfu/g**)*	$6,3*10^6$	$77,5*10^4$	$3,7*10^5$	$5,9*10^4$	$2*10^3$	$1,9*10^3$	$4,7*10^4$
Staphylococcus spp. (cfu/ml/cfu/g**)*	$8,7*10^6$	$7,3*10^6$	$4,4*10^6$	$3,9*10^6$	$2,5*10^6$	$21,5*10^5$	$29,7*10^5$

Conclusions

As a conclusion from this research is that the quality and safety of the dairy products start on the farm level. Therefore, it is important that raw milk be produced and handled properly, because many factors can influence the quality of raw milk such as somatic cells, bacterial contaminations, antibiotics and drugs

residues etc. Results showed significant changes during the 45th day of ripening period, but as a result of milk poor hygienic condition and appearance of mold on cheese surface increases the growth of potential coliform bacteria. The high number of all investigated groups of microorganisms in the raw milk caused unsatisfying results in the final dairy product.

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