

Kalhotka et al. 2013 : 2 (5) 2314-2317

# IMPORTANT GROUPS OF MICROORGANISMS IN RAW GOAT MILK AND FRESH GOAT CHEESES DETERMINED DURING LACTATION

Libor Kalhotka\*<sup>1</sup>, Květoslava Šustová<sup>2</sup>, Michaela Hůlová<sup>1</sup>, Jitka Přichystalová<sup>1</sup>

Address(es): Ing. Libor Kalhotka Ph.D.,

<sup>1</sup>Mendel Univerzity in Brno, Faculty of Agronomy, Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Zemědělská 1, 61300 Brno, Czech Republic, phone number: +420 545 133 028.

<sup>2</sup>Mendel Univerzity in Brno, Faculty of Agronomy, Department of Food Science, Zemědělská 1, 61300 Brno, Czech Republic.

\*Corresponding author: <u>xkalhotk@node.mendelu.cz</u>

ARTICLE INFO

# ABSTRACT

Received 10. 9. 2012 Revised 21. 2. 2013 Accepted 22. 2. 2013 Published 1. 4. 2013

Regular article

The total counts of microorganisms, coliform bacteria, psychrotrophic microorganisms, lactic acid bacteria, enterococci and many others are ranked among the important groups of microorganisms affecting the quality of milk and cheese. In the samples of raw goat milk (farms breeding I and II) collected within 24 h (morning milking - **a**, afternoon milking - **b**) and fresh goat cheese, these groups of microorganisms were determined by standard methods: the total counts of microorganisms (TCM), lactic acid bacteria, coliform bacteria, psychrotrophic microorganisms and enterococci. In cheeses, there was also carried out the determination of yeasts and moulds. After the cultivation, colonies from Petri dishes were counted and the result was expressed in CFU/ml, g. Samples of raw goat milk (except farm II 27.3.) corresponded the requirement of a given legislative act. However, it contained a higher number of coliform and psychrotrophic microorganisms than the stated recommendations. Microbiological analysis showed relatively high as well, they moved between of  $10^3 - 10^8$  CFU/g.

Keywords: microorganisms, goat milk, cheese

### INTRODUCTION

Goat milk is similar in basic composition to cow milk. On average, it contains about 12.6 % of solids, 3.4 % of protein, 3.8 % of fat, 4.3 % of lactose and 0.8 % of minerals (**Herian, 2008**). It can be used for direct consumption or production of various products such as cheese, yogurt and kefir. An important quality factor of goat milk, which one should count with, is microbial contamination.

The total counts of microorganisms (TCM), coliform bacteria, psychrotrophic microorganisms, lactic acid bacteria (LAB), enterococci and many others are ranked among the major groups of microorganisms affecting the quality of milk and cheese.

Total counts of microorganisms in excess of the value of the legislation act tells us about poor hygiene in obtaining milk, insufficient cooling, inconvenient storage of milk and possibility of secondary contamination. Criteria for hygienic quality of goat milk are set in the European Parliament and Council Regulation (EC) No 853/2004. Food business operators must bring up procedures ensuring that total count of microorganisms cultivated at 30 °C would be less than 1 500 000 (per ml) in the raw milk. However, if milk is intended for the manufacture of dairy products from raw milk by a process that does not include any heat treatment, the milk must contain less than 500 000 microorganisms per ml. One of the important group of microorganisms contaminating milk are psychrotrophic microorganisms. The most frequently isolated genera are Pseudomonas, Enterobacter, Flavobacterium. Alcaligenes, Klehsiella. Aeromonas. Acinetobacter, and Achromobacter (Burdová, 1998, Vyletělová et al., 2000b; Chadwick Hayes & Boor, 2001). Many psychrotrophic bacteria isolated from raw milk produce extracellular enzymes that degrade milk proteins and lipids (Vyletělová et al., 2000a). These microorganisms are ubiquitous widespread. Source of their contamination in milk might be, as Cousin (1982) and Burdová (1998) reports, water, soil, air, plants, animals and man.

Coliforms, which are defined as aerobic and facultative anaerobic, asporogenous, gram-negative bacteria that ferment lactose with acid and gas production within 48 h at 32 or 35 °C, include the genera *Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebsiella* (Chadwick Hayes & Boor, 2001). In milk, coliform bacteria are good indicator of primary and secondary contamination. In raw milk, an important group of microorganisms are lactic acid bacteria, the most important genera are *Lactobacillus*, *Lactococcus*, *Leuconostoc*,

Streptococcus, Pediococcus and Enterococcus. According Görner & Valík (2004), they come to milk primarily while grazing in the grass and secondarily from environment where milk is handled. LAB are important in the manufacture of fermented dairy products and cheeses but in raw milk, their activity should be avoided by hygiene compliance and rapid, thorough chilling of milk after milking. Greifová et al. (2003a) reported that enterococci represent a large proportion of autochthon bacteria associated with the mammalian gastrointestinal tract. They often occur in soil, water and on plants. The presence of enterococci in dairy products is referred as an indication of improper sanitation conditions during the milk obtaining and processing. Decisive enterococci contamination of the milk comes from the milking equipment and plant feed, as reported Greifová et al. (2003a).

In goat farms, fresh cheeses are common products. Cheeses, whose production is completed with lactic acid fermentation and salting, are ranked to the group of soft fresh cheese. Produced cheese is of soft consistency (**Grieger**, **1990**). Besides lactic fermentation, there are no other substantial changes in proteins, as **Cempírková** *et al.* (**1997**) reported. For the production of fresh cheese, mesophilic homo- or heterofermentative lactic acid bacteria are suitable. Besides producing lactic acid, aromatic substances especially diacetyl are formed. The bacteria used for production of soft fresh cheese are *Lactococcus lactis* ssp. *lactis, Lactococcus lactis* ssp. *cremoris*, selected according formation of lactic acid with minimal CO<sub>2</sub> production, *Leuconostoc mesenteroides* ssp. *cremoris* and *Leuconostoc mesenteroides* ssp. *destranicum*. In farm-produced cheeses, a number of defects caused by the contaminating microorganisms may occur.

The aim of this study is to compare the microbiological parameters of raw goat's milk and fresh cheese from farms with conventional and organic farming.

# MATERIAL AND METHODS

For microbiological analysis, samples of raw goat milk and fresh goat cheese from farmed animals were used. In the samples of raw goat milk (farms breeding I - conventional farming and II - organic farming) collected within 24 h (morning milking -  $\mathbf{a}$ , afternoon milking -  $\mathbf{b}$ ) and fresh goat cheese, these groups of microorganisms were determined by standard methods: the total counts of microorganisms (TCM) on PCA with skimmed milk (Biokar Diagnostic, France) at 30 °C for 72 h, lactic acid bacteria (LAB) on MRS medium (Biokar Diagnostic, France) at 37 °C for 72 h, coliform bacteria on VRBL medium (Biokar Diagnostic, France) at 37 °C for 24 h, psychrotrophic microorganisms on PCA with skimmed milk (Biokar Diagnostic, France) at 6 °C for 10 days and enterococci on COMPASS ENTEROCOCCUS AGAR (Biokar Diagnostic, France) at 44 °C for 24 h. In cheeses, there was also carried out the determination of yeasts and moulds.

For microbiological analysis of the cheese, the portion was homogenized together with saline solution 1 min. in Stomacher homogenizer. Subsequently, the decimal dilutions series were prepared. Then 1 ml of the dilution was inoculated into sterile Petri dishes and sealed with an appropriate medium. The above-mentioned groups of microorganisms were set. For cheese, the determination of yeasts and moulds was carried out on Chloramphenicol Glucose Agar (Biokar Diagnostics, France) at 25 °C for 120 h. After the cultivation of particular Petri dishes accrued colonies were counted and the result was expressed in CFU/g.

# **RESULTS AND DISCUSSION**

In samples of raw goat milk taken during lactation and fresh goat cheese, the numbers of important groups of microorganisms have been determined. The results of microbiological analysis are presented in Tables 1 to 2.

#### Raw milk

The results show that in raw goat milk, the total number of bacteria for the whole period varied around 10<sup>5</sup> CFU/ml (farm I), with the only one exception resp.  $10^4 - 10^6$  CFU/ml (farm II). Average values then ranged from 1.2 to 2.9 x 10<sup>5</sup> CFU/ml (farm I) resp.  $1.9 \times 10^4$  to  $3.0 \times 10^6$  CFU/ml (farm II). The farm II has supplied pasteurized milk from August to November. The results are not further commented although for completeness, they are shown in Table 1. In farm, incorrect pasteurization or low level of hygiene might be estimated because of high levels of microorganisms ( $10^2 - 10^4$  CFU/ml TCM and  $10^2 - 10^4$  psychrothrophic microorganisms). Similar results were also found out by **Kouřímská & Dvořáková (2008)** who reported a value 1.1 x  $10^5$  CFU/ml as the average value per lactation. EC Regulation No 853/2004 sets limit  $\leq 1500$  000 microorganisms per ml for raw goat milk. It is obvious that the samples of raw milk correspond with the threshold throughout the traced period with some exceptions.

Psychrotrophic bacteria counts ranged from  $10^4 - 10^5$  CFU/ml (farm I) resp.  $10^3 - 10^5$  CFU/ml (farm II). **Görner & Valík (2004)** reported that in milk, the numbers of these bacteria should not exceed 50 000 CFU/ml. In Tab. 1, there is shown that numbers of psychrotrophic bacteria have been detected in milk samples in the range of  $10^3 - 10^5$  CFU/ml and in some cases, the recommended limit was exceeded. Defects of fluid milk, associated with the growth of psychrotrophic bacteria, are related to the production of extracellular enzymes. Sufficient enzyme which causes defects is usually present when the population of psychrotrophs reaches  $10^6$  to  $10^7$  CFU/ml (**Fairbairn & Law, 1987**). Prolongation of pasteurized milk storage by one day is associated with 8.6 mmol/kg increase of FFA (free fatty acids) in the case of psychrotrophic microorganisms occurrence  $10^5 - 10^6$  CFU/ml (**Vyletělová & Hanuš, 2000**).

In samples of raw milk, lactic acid bacteria counts ranged from less than 100 to  $10^5$  CFU/ml. Their negative impact on the quality of raw milk is limited by rapid and thorough chilling in the milk preservation after milking. Coliform bacteria counts ranged from less than 100 to 2.2 x  $10^5$  CFU/ml. **Görner & Valik** (2004) gives a number less than 1 000 CFU/ml as an additional indication of cow milk quality. Taking into consideration the looser limits of EC regulations for goat milk, determined values were not high except some samples. However, there should still be enhanced attention devoted to increased counts of coliform bacteria.

The amount of enterococci ranged from few colonies up to  $10^4$  (farm II) CFU/ml (Table 1). Greifová *et al.* (2003) reported that the decisive enterococci contamination of milk comes from the milking equipment and plant feed. In raw milk, enterococci are clear indicators of inadequate decontamination of equipment and machinery. As thermoresistant bacteria, some species of enterococci survive required pasteurization temperature, therefore they are normal part of pasteurized milk.

Fact, that TCM and psychrotrophic microorganisms in milk from farm I (conventional farming) were very similar during whole lactation, can be clearly seen from the results above. **Callon** *et al.* (2007) reports that the composition of microflora and counts of microorganisms during lactation can be variable and they are involved by many outer factors e.g. physiological state, way of feeding, type of feed and weather. At milk from the second farm (organic farming), higher oscillation of these microorganisms could be suggested. This can be reasoned out from the relatively high counts of microorganisms determined in milk delivered in 8<sup>th</sup> and 11<sup>th</sup> month which was already pasteurized by farmer. Similar trends were found out earlier by Kalhotka *et al.* (2010). Provably higher total counts of microorganisms and coliform bacteria in milk from ecological farming can be seen in Kouřimská *et al.* (2012). Similar results can be expected at goat milk.

		goat milk (	

Date	Sample	ТСМ	goat milk (C	СВ	PM	ENT
27.5.	Ia	2.5 x 10 <sup>5</sup>	2.9 x 10 <sup>5</sup>	2.2 x 10 <sup>5</sup>	2.2 x 10 <sup>5</sup>	1.5 x 10 <sup>3</sup>
	Ib	$9.0 \ge 10^4$	$7.5 \times 10^4$	1.6 x 10 <sup>5</sup>	$5.3 \times 10^4$	$1.4 \times 10^3$
	mean I	1.7 x 10 <sup>5</sup>	1.8 x 10 <sup>5</sup>	1.9 x 10 <sup>5</sup>	1.4 x 10 <sup>5</sup>	1.4 x 10 <sup>3</sup>
	Ha	3.1 x 10 <sup>5</sup>	9.9 x 10 <sup>4</sup>	2.4 x 10 <sup>4</sup>	1.5 x 10 <sup>5</sup>	$1.5 \ge 10^4$
	IIb	1.4 x 10 <sup>5</sup>	1.2 x 10 <sup>5</sup>	4.9 x 10 <sup>4</sup>	$5.7 \times 10^4$	$1.7 \ge 10^4$
	mean II	2.2 x 10 <sup>5</sup>	1.1 x 10 <sup>5</sup>	<b>3.7</b> x 10 <sup>4</sup>	1.0 x 10 <sup>5</sup>	1.6 x 10 <sup>4</sup>
23.6.	Ia	2.6 x 10 <sup>5</sup>	9.9 x 10 <sup>3</sup>	9.4 x 10 <sup>3</sup>	$1.2 \ge 10^4$	$5.6 \times 10^3$
	Ib	2.1 x 10 <sup>5</sup>	$7.0 \ge 10^3$	$1.8 \ge 10^4$	$3.5 \times 10^4$	$2.8 \times 10^3$
	mean I	2.3 x 10 <sup>5</sup>	8.5 x 10 <sup>3</sup>	1.4 x 10 <sup>4</sup>	2.4 x 10 <sup>4</sup>	4.2 x 10 <sup>3</sup>
	Ha	$5.0 \ge 10^4$	< 100	$2.5 \times 10^2$	$2.0 \times 10^3$	< 10
	IIb	$1.4 \text{ x } 10^4$	< 100	$1.0 \ge 10^2$	2.7 x 10 <sup>3</sup>	< 10
	mean II	3.2 x 10 <sup>4</sup>	< 100	1.8 x 10 <sup>2</sup>	$2.4 \times 10^3$	< 10
23.7.	Ia	1.3 x 10 <sup>5</sup>	$1.1 \ge 10^3$	< 100	$2.1 \times 10^4$	< 10
	Ib	1.1 x 10 <sup>5</sup>	$2.0 \ge 10^2$	< 100	$1.6 \ge 10^4$	< 10
	mean I	1.2 x 10 <sup>5</sup>	6.7 x 10 <sup>2</sup>	< 100	<b>1.9 x 10</b> <sup>4</sup>	< 10
	IIa	$2.6 \ge 10^{6}$	$2.0 \ge 10^4$	1.1 x 10 <sup>5</sup>	$1.9 \ge 10^4$	$6.2 \times 10^3$
	IIb	$3.5 \ge 10^{6}$	$1.9 \ge 10^4$	1,0 x 10 <sup>5</sup>	$1.9 \ge 10^4$	$9.6 \ge 10^3$
	mean II	3.0 x 10 <sup>6</sup>	1.9 x 10 <sup>4</sup>	1.1 x 10 <sup>5</sup>	1.9 x 10 <sup>4</sup>	$7.9 \ge 10^3$
5.8.	Ia	3.1 x 10 <sup>5</sup>	$4.4 \times 10^3$	$1.4 \ge 10^4$	3.1 x 10 <sup>5</sup>	$2.8 \times 10^3$
	Ib	$2.7 \times 10^5$	$1.3 \times 10^4$	$9.2 \times 10^4$	$5.9 \times 10^4$	$3.4 \ge 10^3$
	mean I	2.9 x 10 <sup>5</sup>	8.8 x 10 <sup>3</sup>	5.3 x 10 <sup>4</sup>	1.8 x 10 <sup>5</sup>	3.1 x 10 <sup>3</sup>
	IIa*	$2.5 \ x \ 10^4$	$2.3 \times 10^2$	50	$8.6 \ge 10^3$	< 10
	IIb*	$1.4 \ge 10^4$	91	$1.5 \ge 10^2$	$3.1 \ge 10^4$	< 10
	mean II	1.9 x 10 <sup>4</sup>	1.6 x 10 <sup>2</sup>	1.0 x 10 <sup>2</sup>	2.0 x 10 <sup>4</sup>	< 10
13.10.	Ia	1.4 x 10 <sup>5</sup>	$3.9 \times 10^3$	$6.2 \times 10^3$	$4.5 \ge 10^4$	$4.5 \times 10^2$
	Ib	1.3 x 10 <sup>5</sup>	$4.1 \ge 10^3$	2.1 x 102	$2.4 \times 10^4$	$3.0 \ge 10^2$
	mean I	1.3 x 10 <sup>5</sup>	4.0 x 10 <sup>3</sup>	3.2 x 10 <sup>3</sup>	3.4 x 10 <sup>4</sup>	3.8 x 10 <sup>2</sup>
	IIa*	$3.2 \times 10^4$	< 100	3	$8.2 \times 10^3$	1
	IIb*	$1.4 \ge 10^4$	< 100	8	$9.5 \ge 10^3$	1
	mean II	2.3 x 10 <sup>4</sup>	< 100	6	8.9 x 10 <sup>3</sup>	1
10.11.	Ia	1.6 x 10 <sup>5</sup>	$2.4 \times 10^4$	$1.6 \ge 10^3$	1.9 x 10 <sup>4</sup>	$6.8 \ge 10^2$
	Ib	2.3 x 10 <sup>5</sup>	$2.7 \text{ x } 10^4$	$1.7 \text{ x } 10^3$	$2.7 \text{ x } 10^4$	$1.0 \ge 10^3$
	mean I	1.9 x 10 <sup>5</sup>	2.6 x 10 <sup>4</sup>	1.6 x 10 <sup>3</sup>	2.3 x 10 <sup>4</sup>	8.5 x 10 <sup>2</sup>
	IIa*	$2.8 \times 10^2$	2	2	$1.5 \ge 10^2$	ND
	IIb*	69	3	2	$1.0 \ge 10^2$	ND
	mean I	$1.7 \times 10^2$	2	2	1.3 x 10 <sup>2</sup>	ND
I egend.	* nasteu	rized milk	ND – not	detected T	CM - total	count of

**Legend:** \* pasteurized milk, ND – not detected, TCM – total count of microorganisms, LAB – lactic acid bacteria, CB – coliform bacteria, PM – psychrotrophic microorganisms, ENT - enterococci

#### Fresh goat cheese

Together with the samples of milk, cheeses from both farms were delivered for analysis. Table 2 presents the results of microbiological analysis of fresh goat cheese made from analyzed milk. The results of microbiological analysis from Table 2 show that the total number of bacteria in cheese ranged in orders of magnitude  $10^7 - 10^8$  CFU/g (farm I) resp.  $10^8 - 10^9$  CFU/g (farm II). Results cannot be attributed only with indication of the low level of hygiene in the production of cheese, but there are significantly reflected lactic acid bacteria whose numbers have varied in the range between  $10^7 - 10^8$  CFU/g. These bacteria are the most important group of microorganisms from the technological point of view.

			goat cheeses (	CFU/g) Coliform	Psychrotroph.					
Date	Sample	ТСМ	LAB	bac.	m.	Enterococci	Total counts	Yeasts Yeasts	and moulds Moulds	
									Total counts	Geotrichum
27.5.	Ia	2.7 x 10 <sup>7</sup>	7.0 x 10 <sup>7</sup>	1.4 x 10 <sup>4</sup>	1.2 x 10 <sup>4</sup>	1.1 x 10 <sup>3</sup>	25	10	15	ND
	Ib	4.8 x 10 <sup>8</sup>	6.0 x 10 <sup>7</sup>	2.0 x 10 <sup>4</sup>	$2.7 \times 10^4$	$1.2 \times 10^2$	50	0	50	50
	mean I	2.5 x 10 <sup>8</sup>	<b>3.4</b> x 10 <sup>7</sup>	<b>1.7 x 10<sup>4</sup></b>	<b>2.0</b> x $10^4$	5.9 x 10 <sup>2</sup>	38	5	33	25
	IIa	5.9 x 10 <sup>8</sup>	4.6 x 10 <sup>8</sup>	3.9 x 10 <sup>6</sup>	$2.5 \times 10^4$	$8.7 \times 10^3$	$7.7 \times 10^3$	$6.3 \times 10^3$	$1.4 \ge 10^3$	$1.4 \times 10^3$
	IIb	3.1 x 10 <sup>8</sup>	3.6 x 10 <sup>8</sup>	1.3 x 10 <sup>6</sup>	$2.7 \times 10^4$	$1.0 \ge 10^4$	$9.0 \ge 10^3$	$7.8 \times 10^3$	$1.2 \times 10^3$	$1.2 \times 10^3$
	mean II	4.5 x 10 <sup>8</sup>	4.1 x 10 <sup>8</sup>	2.6 x 10 <sup>6</sup>	<b>2.6</b> x 10 <sup>4</sup>	9.5 x 10 <sup>3</sup>	8.3 x 10 <sup>3</sup>	7.0 x $10^3$	1.3 x 10 <sup>3</sup>	1.3 x 10 <sup>3</sup>
23.6.	Ia	1.5 x 10 <sup>8</sup>	$1.6 \ge 10^7$	$6.4 \times 10^2$	$5.9 \times 10^3$	ND	ND	ND	ND	ND
	Ib	9.8 x 10 <sup>7</sup>	$4.0 \ge 10^7$	$1.2 \times 10^4$	$3.6 \times 10^3$	$1.4 \times 10^3$	ND	ND	ND	ND
	mean I	1.3 x 10 <sup>8</sup>	<b>2.8</b> x 10 <sup>7</sup>	6.2 x 10 <sup>3</sup>	<b>4.8</b> x 10 <sup>3</sup>	7.2 x $10^2$	ND	ND	ND	ND
	IIa	4.9 x 10 <sup>8</sup>	3.6 x 10 <sup>8</sup>	1.8 x 10 <sup>6</sup>	$2.2 \times 10^7$	5.2 x 10 <sup>6</sup>	$1.2 \ge 10^4$	1.2 x 10 <sup>4</sup>	ND	ND
	IIb	3.0 x 10 <sup>8</sup>	2.5 x 10 <sup>8</sup>	1.1 x 10 <sup>7</sup>	1.0 x 10 <sup>7</sup>	4.0 x 10 <sup>6</sup>	$3.8 \times 10^3$	3.8 x 10 <sup>3</sup>	ND	ND
	mean II	<b>3.9</b> x 10 <sup>8</sup>	3.0 x 10 <sup>8</sup>	6.6 x 10 <sup>6</sup>	<b>1.6 x 10<sup>7</sup></b>	4.6 x 10 <sup>6</sup>	7.7 x $10^3$	7.7 x 10 <sup>3</sup>	ND	ND
23.7.	Ia	6.8 x 10 <sup>8</sup>	2.3 x 10 <sup>8</sup>	1.3 x 10 <sup>4</sup>	7.7 x 10 <sup>4</sup>	20	78	55	23	ND
	Ib	3.5 x 10 <sup>8</sup>	$1.5 \ge 10^7$	1.4 x 10 <sup>4</sup>	$1.2 \times 10^4$	ND	10	5	5	ND
	mean I	5.1 x 10 <sup>8</sup>	1.2 x 10 <sup>8</sup>	1.3 x 10 <sup>4</sup>	4.5 x 10 <sup>4</sup>	10	44	30	14	ND
	IIa	7.5 x 10 <sup>8</sup>	1.3 x 10 <sup>8</sup>	6.2 x 10 <sup>5</sup>	$3.3 \times 10^{6}$	6.1 x 10 <sup>6</sup>	1.2 x 10 <sup>5</sup>	1.2 x 10 <sup>5</sup>	45	45
	IIb	5.0 x 10 <sup>8</sup>	1.8 x 10 <sup>8</sup>	7.6 x 10 <sup>5</sup>	8.7 x 10 <sup>6</sup>	2.3 x 10 <sup>6</sup>	1.9 x 10 <sup>5</sup>	1.9 x 10 <sup>5</sup>	ND	ND
	mean II	6.3 x 10 <sup>8</sup>	1.5 x 10 <sup>8</sup>	6.9 x 10 <sup>5</sup>	6.0 x 10 <sup>6</sup>	4.2 x 10 <sup>6</sup>	1.6 x 10 <sup>5</sup>	1.6 x 10 <sup>5</sup>	23	23
5.8.	Ia	4.1 x 10 <sup>8</sup>	2.9 x 10 <sup>7</sup>	$2.3 \times 10^2$	$3.2 \times 10^4$	ND	5	5	ND	ND
	Ib	2.2 x 10 <sup>8</sup>	3.6 x 10 <sup>7</sup>	1.7 x 10 <sup>4</sup>	$2.0 \times 10^5$	25	32	27	5	ND
	mean I	3.1 x 10 <sup>8</sup>	<b>3.3</b> x 10 <sup>7</sup>	8.7 x 10 <sup>3</sup>	<b>1.2</b> x 10 <sup>5</sup>	13	19	16	3	ND
	IIa	3.4 x 10 <sup>8</sup>	3.2 x 10 <sup>7</sup>	1.2 x 10 <sup>7</sup>	4.9 x 10 <sup>6</sup>	4.0 x 10 <sup>6</sup>	1.6 x 10 <sup>5</sup>	1.6 x 10 <sup>5</sup>	45	ND
	IIb	1.9 x 10 <sup>8</sup>	4.4 x 10 <sup>7</sup>	7.4 x 10 <sup>6</sup>	3.7 x 10 <sup>6</sup>	3.6 x 10 <sup>6</sup>	2.3 x 10 <sup>5</sup>	2.3 x 10 <sup>5</sup>	0	ND
	mean II	2.7 x 10 <sup>8</sup>	<b>3.8</b> x 10 <sup>7</sup>	9.8 x 10 <sup>6</sup>	4.3 x 10 <sup>6</sup>	<b>3.8</b> x 10 <sup>6</sup>	1.9 x 10 <sup>5</sup>	1.9 x 10 <sup>5</sup>	23	ND
13.10.	Ia	6.1 x 10 <sup>8</sup>	2.7 x 10 <sup>8</sup>	50	5.7 x 10 <sup>5</sup>	$6.3 \times 10^2$	5	ND	5	ND
	Ib	1.7 x 10 <sup>8</sup>	4.5 x 10 <sup>7</sup>	$3.0 \ge 10^2$	3.6 x 10 <sup>5</sup>	23	5	ND	5	ND
	mean I	3.9 x 10 <sup>8</sup>	1.6 x 10 <sup>8</sup>	1.8 x 10 <sup>2</sup>	<b>4.7</b> x 10 <sup>5</sup>	3.3 x 10 <sup>2</sup>	5	ND	5	ND
	IIa*	1.5 x 10 <sup>9</sup>	6.0 x 10 <sup>8</sup>	$1.0 \ge 10^2$	1.5 x 10 <sup>8</sup>	5.2 x 10 <sup>7</sup>	5.2 x 10 <sup>5</sup>	5.2 x 10 <sup>5</sup>	50	ND
	IIb*	6.2 x 10 <sup>8</sup>	2.1 x 10 <sup>8</sup>	$1.5 \ge 10^2$	3.2 x 10 <sup>6</sup>	$4.3 \times 10^7$	5.4 x 10 <sup>5</sup>	5.4 x 10 <sup>5</sup>	ND	ND
	mean II	1.1 x 10 <sup>9</sup>	4.1 x 10 <sup>8</sup>	1.3 x 10 <sup>2</sup>	<b>7.7 x 10<sup>7</sup></b>	<b>4.8</b> x 10 <sup>7</sup>	5.3 x 10 <sup>5</sup>	5.3 x 10 <sup>5</sup>	25	ND
10.11.	Ia	5.7 x 10 <sup>8</sup>	7.4 x 10 <sup>7</sup>	1.1 x 10 <sup>3</sup>	$1.2 \times 10^4$	1.4 x 10 <sup>4</sup>	5.7 x 10 <sup>2</sup>	5.6 x 10 <sup>2</sup>	10	ND
	Ib	3.8 x 10 <sup>8</sup>	1.0 x 10 <sup>8</sup>	1.2 x 10 <sup>5</sup>	$6.0 \ge 10^3$	15	20	15	5	ND
	mean I	4.7 x 10 <sup>8</sup>	<b>8.9 x 10<sup>7</sup></b>	5.9 x 10 <sup>4</sup>	8.8 x 10 <sup>3</sup>	6.8 x 10 <sup>3</sup>	<b>2.9</b> x 10 <sup>2</sup>	$2.9 \times 10^2$	8	ND
	IIa*	2.2 x 10 <sup>8</sup>	1.6 x 10 <sup>7</sup>	< 100	2.3 x 10 <sup>6</sup>	1.6 x 10 <sup>7</sup>	2.9 x 10 <sup>4</sup>	2.9 x 10 <sup>4</sup>	ND	ND
	IIb*	1.9 x 10 <sup>8</sup>	2.1 x 10 <sup>7</sup>	< 100	$2.9 \times 10^{6}$	$1.7 \times 10^7$	$2.0 \times 10^4$	$2.0 \times 10^4$	$2.7 \times 10^2$	$2.7 \times 10^2$
	mean II	2.1 x 10 <sup>8</sup>	<b>1.8</b> x 10 <sup>7</sup>	< 100	<b>2.6 x 10<sup>6</sup></b>	<b>1.6 x 10<sup>7</sup></b>	2.5 x 10 <sup>4</sup>	2.5 x 10 <sup>4</sup>	$1.4 \times 10^2$	$1.4 \times 10^2$
Laga					LAB – lactic acid bact		210 A 1V	2.0 A 10	1.1 A 10	1.1 A 10

Legend: \*Goat gervais, TCM - total count of microorganisms, LAB - lactic acid bacteria

In all samples of cheese, coliform bacteria were detected. Their numbers have varied from several tens to  $1.2 \times 10^5$  CFU/g (farm I) resp.  $1.2 \times 10^7$  CFU/g (farm II). ČSN 56 9609 for fresh cheese gives the limit of  $5 \times 10^2$  resp.  $2 \times 10^3$  CFU/g. The occurrence of these bacteria indicates a low level of hygiene in the production of cheese. These bacteria are the cause of the defects of fresh cheeses. Approximately  $10^7$  CFU of coliform per gram is needed to produce a gassy defect (Frank, 2001). They are also producers of biogenic amines (Kalhotka *et al.*, 2012a) and some of them may also be pathogenic for humans. The numbers of

psychrotrophic microorganisms which varied within the order of magnitude  $10^3 - 10^5$  CFU/g (farm I) resp.  $10^4 - 10^8$  CFU/g (farm II) also indicates the possibility of secondary contamination in cheese production.

At the first sight, enterococci act contradictory in food microbiology. In fermented foods, they have probiotic properties and they are able to form bacteriocins and needed for cheese ripening (Greifová *et al.*, 2003). The presence of enterococci in dairy products is also referred as an indication of lack of sanitation conditions during the milk obtaining and processing. Enterococci are

also important producers of biogenic amines especially tyramine (Halász *et al.*, 1994; Bover-Cid & Holzapfel, 1999; Greifová *et al.*, 2003b and Kalhotka *et al.*, 2012b). Counts of enterococci ranged from the threshold of detection to  $10^4$  CFU/g (farm I) resp.  $10^3 - 10^7$  CFU/g (farm II). In traditional and artisanal cheeses produced using raw milk, enterococci usually reach  $10^7$  CFU/g (Wessels *et al.*, 1990). Numbers of enterococci in Mediterranean-type cheese curds range from  $10^4$  to  $10^6$  CFU/g, and in the fully ripened cheeses from  $10^5$  to  $10^7$  CFU/g. (Franz *et al.*, 2003)

During cheese production, contamination by yeasts and moulds can result in final product. Growth of yeasts and moulds is a common cause of spoilage of fermented dairy products, because these microorganisms are able to grow well at a low pH. Yeast spoilage is manifested as fruity or yeasty odor and/or gas formation (Frank, 2001). This group of microorganisms occurred in cheese but their numbers were low (farm I) being dominated by the yeasts. However, high counts of molds and yeast were detected in farm II in orders of magnitude  $10^{\circ}$ CFU/g. There were higher counts of yeast compare to molds again. Counts of yeast did not increase the limits 107 given by ČSN 569609. Görner & Valík (2004) reported that yeasts significantly grow on surface of packed cheese. If the package is ruptured, fungal growth, usually genera Penicillium and Aspergillus who dominates in the air of cheese diaries (Johnson, 2001), can occur there. Cheese is a frequently contaminated by Geotrichum because it commonly inhabits raw milk and it is part of natural microflora in fresh cheese made of goat milk (Hudecová et al., 2008). The cheese from pasteurized milk is the most likely source of its contamination from the environment. However, the cheese analysis showed the appearance of Geotrichum in only one sample (farm I) resp. 4 in farm II (see Table 2). On cheese, there were no apparent increases of moulds. Cheese from farm II was worse quality, unlikely taste and there was a smell of veast.

#### CONCLUSION

Samples of raw goat milk (except farm II 23. 7.) corresponded with the requirement of a given legislative act. However, it contained a higher number of coliform and psychrotrophic microorganisms than the stated recommendations. Pasteurization of milk was a reason of significant reduction of microbial contamination by several orders of magnitude or it was on threshold of detection.

Microbiological analysis showed that for all cheeses, numbers of unwanted coliform bacteria were relatively high. Counts of psychrotrophic microorganisms were relatively high as well. This indicates a low level of hygiene in the production of cheese at the farm. Although in some samples of milk produced by organic farming, the microbial contamination was lower than in some samples of milk produced by conventional farming, cheeses made from this milk were evaluated oppositely. Cheeses from farm II (organic farming) had significantly worse quality. In conclusion, it is necessary to pay maximum attention to hygiene during milk obtaining and cheese manufacturing.

Acknowledgments: The project was supported by the Ministry of Education project 2B08069 National Research Program - NRP II Programme 2B - Healthy and Quality Life – Research of relationships between the characteristics of contaminant microflora and production of biogenic amines as risk toxicants in the system of wholesomeness assessment in cheeses in the consumer market.

## REFERENCES

BOVER-CID, S., HOLZAPFEL, W.H. 1999. Improved screening procedure for biogenic amine production by lactic acid bacteria. *Int. J. of Food Microbiology*, 53, 33-41.

BURDOVÁ, O. 1998. Kvalita mliečnych výrobkov v závislosti od mikrobiálnej kontaminácie surového mlieka. *Mliekarstvo*, 4, 44-45.

CALLON, C., DUTHOIT, F., DELBÈS, C., FERRAND, M., LE FRILEUX, Y., DE CRÈMOUX, R., MONTEL, M.C. 2007. Stability of microbial communities in goat milk during a lactation year: Molecular approaches. *Syst. and Appl. Microbiology*, 30, 547-560.

CEMPÍRKOVÁ, R., LUKÁŠOVÁ, J., HEJLOVÁ, Š. 1997. Mikrobiologie potravin. Scriptum JU ZF České Budějovice: 165 p. ISBN 80-7040-254-7.

CHADWICK HAYES, M., BOOR, K. 2001. Raw milk and fluid milk products. In: Marth, E. H., Steele, J. L. (eds.) *Applied Dairy Microbiology*. Marcel Dekker Inc, New York, USA: 744 p. ISBN 0-8247-0536-X.

COUSIN, M.A. 1982. Presence and activity of psychrothrophic microorganisms in milk and dairy products: a review. *J. Foot. Prot.*, 45, p. 172 – 207. In: Frank, J. F. 2001. *Milk and Dairy Products*. In: Doyle, M. P., Beuchat, L. R., Montville, T. J. (eds.) *Food Microbiology*, Fundamentals and Frontiers. ASM Press, Washington DC, USA: 872 p. ISBN 1-55581-208-2.

ČSN 56 9609. 2008. Pravidla správné hygienické a výrobní praxe – mikrobiologická kritéria pro potraviny. Principy stanovení a aplikace.

EUROPEAN PARLIAMENT AND COUNCIL REGULATION (EC) No 853/2004.

FAIRBAIRN D.J., LAW B. A. 1987. The effect of nitrogen and carbon sources on proteinase production by Pseudomonas fluorescens. *J. Appl. Bacteriol.*, 62, p. 105-113. In: Frank, J. F. 2001. *Milk and Dairy Products*. In: Doyle, M. P., Beuchat, L. R., Montville, T. J. (eds.) *Food Microbiology*: Fundamentals and Frontiers. ASM Press, Washington DC, USA: 872 p. ISBN 1-55581-208-2.

FRANK, J. F. 2001. Milk and Dairy Products. In: Doyle, M.P., Beuchat, L.R., Montville, T. J. (eds.) *Food Microbiology*: Fundamentals and Frontiers. ASM Press, Washington DC, USA: 872 p. ISBN 1-55581-208-2.

FRANZ, CH.M.A.P., STILES, M.E., SCHLEIFER, K.H., HOLZAPFEL, W.H. 2003. Enterococci in foods – a conundrum for food safety. *Int. J. of Food Microbiology*, 88, 105-122.

GÖRNER, F., VALÍK, Ľ. 2004. Aplikovaná mikrobiológia požívatín. Malé centrum, Bratislava: 528 p. ISBN 80-967064-9-7.

GREIFOVÁ, M., GREIF, G., LEŠKOVÁ, E., MÉRIOVÁ, K. 2003a. Enterokoky – ich hodnotenie v mliekarenskej technológii. *Mliekarstvo*, 2, 42-45. GREIFOVÁ, M., GREIF, G., NOVOROĽNÍKOVÁ, B., KUBOVÁ, A. 2003b. Biogénne amíny v mliečnych výrobkov a ich tvorba enterokokami. *Mliekarsvo*, 4, 31-33.

GRIEGER, C. 1990. Ovčie a kozie mlieko a výrobky z nich. In: Grieger, C., Holec, J. (eds.) *Hygiena mlieka a mliečnych výrobkov*, Príroda : Bratislava 397 p. ISBN 80-07-00253-7.

HALÁSZ, A., BARÁTH, A., SIMON-SAKARDI, L., HOLZAPFEL, W. 1994. Biogenic amines and their production by microorganisms in food. *Trends in Food Science Technology*, 5, 42-49.

HERIAN, K. 2008. Ovčie a kozie mliekarstvo na Slovensku. Farmářská výroba sýrů a kysaných mléčných výrobků V. *Sborník referátů ze semináře s mezinárodní účastí*, Brno, 38-44.

HUDECOVÁ, A., LIPTÁKOVÁ, D., VALÍK, Ľ., MEDVEĎOVÁ, A. 2008. Kvantifikácia rastu *Geotrichum candidum* v mlieku: vplyv teploty a pH. *Mliekarstvo*, 3, 25-29.

JOHNSON, M.E. 2001. Cheese Product. In: Marth EH, Steele JL, (eds.) Applied Dairy Microbiology. Marcel Dekker Inc, New York, USA: 744 p. ISBN 0-8247-0536-X.

KALHOTKA, L., ŠUSTOVÁ, K., KVASNIČKOVÁ, B., LUŽOVÁ, T., HAVLÍKOVÁ, Š. 2010. Změny mikroflóry syrového kozího mléka v průběhu laktace. *Mlékařské listy Zpravodaj*, 119, 14-17.

KALHOTKA, L., MANGA, I., HŮLOVÁ, M., PŘICHYSTALOVÁ, J., LUŽOVÁ, T. 2012. Testování dekarboxylasové aktivity *Escherichia coli*. In: Čurda, L., Štětina, J. Celostátní přehlídky sýrů 2012 Výsledky přehlídek a sborník přednášek konference Mléko a sýry. Technická 5, 166 28 Praha 6: Vysoká škola chemicko-technologická v Praze, s. 160-164.

KALHOTKA, L., MANGA, I., PŘICHYSTALOVÁ, J., HŮLOVÁ, M., VYLETĚLOVÁ, M., ŠUSTOVÁ, K. 2012b. Decarboxylase activity test of the genus Enterococcus isolated from goat milk and cheese. *Acta Veterinaria Brno*, 81(2), 145-151.

KOUŘIMSKÁ, L., DVOŘÁKOVÁ, B. 2008. Kvalita kozího mléka v průběhu laktačního období. *Sborník z konference Den mléka 2008*, 23.9. ČZU v Praze, 108-109.

KOUŘIMSKÁ, L., LEGAROVÁ, V., HERING, P. 2012. Porovnání kvality mléka z konvenčního a ekologického zemědělství. In: Čurda, L., Štětina, J. Celostátní přehlídky sýrů 2012. Výsledky přehlídek a sborník přednášek konference Mléko a sýry. Technická 5, 166 28 Praha 6: Vysoká škola chemicko-technologická v Praze, 44-48.

VYLETĚLOVÁ, M., FICNAR, J., HANUŠ, O. 2000a. Effects of lipolytic enzymes *Pseudomonas fluorescens* on liberation of fatty acids from milk fat. *Czech J. Food Sci.*, 18(5), 175-182.

VYLETĚLOVÁ, M., HANUŠ, O. 2000. Effects of contamination by *Pseudomonas fluorescens* on principal components and technological parameters of pasteurized milk during storage. *Czech J. Food Sci.*, 18(6), 224 - 234.

VYLETĚLOVÁ, M., HANUŠ, O., URBANOVÁ, E., KOPUNECZ, P. 2000b. The occurence and identification of psychrotrophic bacteria with proteolytic and lipolytic activity in bulk milk samples at storage in primary production conditions. *Czech J. Animal Sci.*, 45, 373-383.

WESSELS, D., JOOSTEN, P.J., MOSTERT, J.F. 1990. Technologically important characteristic of *Enterococcus* isolates from milk and dairy products. *International Food Microbiology*, 10, 349-352.