ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 67 60 Number 3, 2019

COMPARISON OF MICROBIOLOGICAL PARAMETERS OF FRESH GOAT CHEESES PRODUCED ON FARMS IN THE CZECH REPUBLIC USING CONVENTIONAL AND ORGANIC FARMING METHOD

Libor Kalhotka[1], Jitka Přichystalová[1], Lenka Dostálová[1],
Květoslava Šustová[2], Michaela Hůlová[1], Eva Burdová[1], Jan Kuchtík[3]

[1]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1/1665, 613 00 Brno, Czech Republic
[2]Department of Food Technology, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1/1665, 613 00 Brno, Czech Republic
[3]Department of Animal Breeding, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1/1665, 613 00 Brno, Czech Republic

To link to this article: https://doi.org/10.11118/actaun201967030695
Received: 8. 1. 2019, Accepted: 15. 4. 2019

To cite this article: KALHOTKA LIBOR, PŘICHYSTALOVÁ JITKA, DOSTÁLOVÁ LENKA, ŠUSTOVÁ KVĚTOSLAVA, HŮLOVÁ MICHAELA, BURDOVÁ EVA, KUCHTÍK JAN. 2019. Comparison of Microbiological Parameters of Fresh Goat Cheeses Produced on Farms in the Czech Republic Using Conventional and Organic Farming Method. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 67(3): 695–704.

Abstract

The paper deals with microbiological parameters of goat cheeses produced on two farms during several years. The first farm used conventional farming method with about 130 dairy goats and the second one applied organic farming method with approximately 500 dairy goats. In samples of fresh goat cheese taken in four years, there were determined the following groups of microorganisms: total count of microorganisms, lactic acid bacteria, coliform bacteria, psychrotrophic microorganisms, enterococci and micromycetes. The comparison of the average numbers of individual groups of microorganisms in cheeses showed a statistically significant difference between the farms. Microbiological quality was found worse at cheeses manufactured at the farm using organic farming method, compared with cheeses from the conventional farm. Higher numbers of coliforms, psychrotrophic microorganisms, enterococci and fungi were more frequently detected in cheeses from the organic farm. Worse microbiological quality of the cheese was also reflected in sensory properties, especially the smell, colour and consistency which was evident when preparing individual samples.

Keywords: coliform bacteria, lactic acid bacteria, psychrotrophic microorganisms, total count of microorganisms, cheese, milk

INTRODUCTION

In the last two decades, the demand for goat's milk and its dairy products has been constantly increasing, especially because of its better digestion and less allergenic potential. Consumers are more interested in the products also due to the traditional method of producing goat cheese in connection with their specific sensory properties (Masotti *et al.*, 2012; El Galiou *et al.*, 2015). However, these products represent a suitable environment for microbial growth whose activities can lead to significant financial loss or health hazards.

Cheese production is complex process where not only milk but also different stages of production must meet high technological and hygienic demands. The milk may not contain pathogenic microorganisms. Inappropriate milk is also the milk contaminated with large amounts of psychrotrophic microorganisms, coliform bacteria and butyric acid bacteria. Raw milk may contain dangerous contaminating pathogens such as *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, and *Clostridium perfringens*. For these reasons, the milk intended must be pasteurized.

Assortment of cheeses produced on farms rearing goats and sheep continues to expand with increasing experience of farmers, technological equipment and consumers demand. Our farmers offer ripened cheeses (semi-hard and hard cheeses), interior ripened cheese, and cheese with mould (Dragounová and Toušová, 2008). However, the most common product on the farms is fresh cheese.

Cheese manufactured at a farm may exhibit a number of defects caused by the activities of contaminating microorganisms caused by secondary contamination of the milk during production of cheese, or originated from inadequately pasteurized milk.

Farm cheeses, which are delivered to the market, are subjected to inspection and state supervision and there are limits given by the Commission Regulation (EC) No. 1441/2007. There are listed following parameters for a particular category: *Salmonella* and *Listeria monocytogenes* must not be present in 25 g of the product, counts of coagulase-positive staphylococci vary in particular categories within the range from tens to 10^5 CFU.g^{-1} and *E. coli* from 10^2 to 10^3 CFU.g^{-1}. For evaluating the microbiology of cheese, data from ČSN 569609 can also be used, but those are not legally binding. The standard divides cheese into several categories and determines similar microbiological parameters within the category. Within individual categories of cheeses, tolerable values of coliforms range from 10^2 to 10^5 CFU.g^{-1}, *E. coli* from tens to 10^4 CFU.g^{-1}, coagulase-positive staphylococci may be present from 10^3 to 10^4 CFU.g^{-1}, micromycetes up to 10^3 CFU.g^{-1} and *Listeria monocytogenes* may not occur in 25 g of the cheese.

The aim of the paper was to compare the microbiological parameters of fresh goat cheeses produced on two farms in the Czech Republic using conventional and organic farming.

MATERIALS AND METHODS

Over several years, unflavoured fresh goat cheeses made from pasteurized milk produced on two farms (A and B) were monitored and analysed. The technological process of cheese production on farms was similar. The cheeses were shipped in retail packaging to the market. Farm A bred white shorthair goats (about 130 dairy goats) and used conventional farming. B farm bred white shorthair goats (550 dairy goats) using organic farming. Other selected parameters are listed in Tab. I.

Sampling was performed once a month during lactation. Two freshly produced samples of cheese stored in a consumer package and two samples of milk (250 ml) were aseptically collected and transported to the laboratory carried in isolated containers at 4 ± 1 °C. Samples of raw goat milk were collected within 24 h (afternoon and morning milking). After delivery, the samples were immediately processed in the laboratory and analysed. For microbiological analysis of the cheese, the sample of cheese (10 g) was homogenized together with saline solution 1 min. in Stomacher homogenizer. Subsequently, the decimal dilutions series were prepared. Afterwards, 1 ml of the dilution was inoculated into sterile Petri dishes and sealed with an appropriate medium.

I: *Selected parameters relating to milking on farms and farm products*

Farm	Milking	Toilet m. gland	Parlor	Milking post-treatment	Cooling equipment	Products
A	2×	wet	parallel	no	kettle	milk, cheeses
B	2×	wet	parallel	barrier	kettle	milk, cheeses, yogurts, whey drinks

In samples of cheese, the following groups of microorganisms were determined using standard methods: the total counts of microorganisms (TCM) on PCA with skimmed milk (Biokar Diagnostics, France) at 30 °C for 72 h, the lactic acid bacteria on MRS agar (Biokar Diagnostics, France) at 37 °C for 72 h, coliforms on VRBL (Biokar Diagnostics, France) at 37 °C for 24 h, psychrotrophic microorganisms on PCA with skimmed milk at 6 °C for 10 days, enterococci on COMPASS ENTEROCOCCUS Agar (Biokar Diagnostics, France) at 44 °C for 24 h, micromycetes on Chloramphenicol Glucose Agar (Biokar Diagnostics, France) at 25 °C for 120 h. TCM was detected within microbiological analysis of raw milk used for cheese production. Afterwards, typical colonies were counted and the result was expressed as CFU per 1 g or ml. Statistical analysis was performed using Microsoft Excel and STATISTICA 12 (StatSoft) – one-way analysis of variance (ANOVA) followed by evaluation using Tukey test with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Although farms producing cheese differ in farming methods and in the number of dairy goats, milking technology and processing of milk were very similar in the monitored period. Quality of raw milk was satisfactory. With some exceptions, TCM did not exceed the limit set by the European Parliament and Council Regulation (EC) No. 853/2004. The results of microbiological analysis for the reported period are presented in Tab. II. Similar TCM in raw milk was reported by Delgado-Pertiñez *et al.* (2003), Morgan *et al.* (2003) and Cupáková *et al.* (2006). In both farms, cheese

II: *The mean values of TCM (from evening and morning milking) in raw goat milk produced on conventional farm (A) and organic farm (B) during the monitored period in CFU.ml^{-1}*

Year	Month	Farm A	Farm B
I.	**VII.**	4.2×10^5	1.1×10^5
	VIII.	2.8×10^5	1.2×10^5
	IX.	1.3×10^5	3.9×10^6
	X.	4.7×10^4	2.3×10^5
	XI.	1.2×10^5	–
	mean	$\mathbf{1.8 \times 10^5}$	$\mathbf{1.1 \times 10^6}$
II.	**V.**	2.0×10^5	$1{,}4 \times 10^5$
	VI.	2.2×10^5	–
	VII.	1.3×10^5	–
	VIII.	2.3×10^5	1.4×10^5
	IX.	9.3×10^4	–
	XI.	2.5×10^5	6.8×10^4
	mean	$\mathbf{1.8 \times 10^5}$	$\mathbf{1.3 \times 10^5}$
III.	**V.**	1.7×10^5	2.2×10^5
	VI.	2.3×10^5	3.2×10^4
	VII.	1.2×10^5	3.0×10^6
	VIII.	2.9×10^5	–
	X.	1.3×10^5	–
	XI.	1.9×10^5	–
	mean	$\mathbf{1.9 \times 10^5}$	$\mathbf{1.1 \times 10^6}$
IV.	**V.**	6.3×10^4	–
	VI.	1.2×10^5	6.8×10^5
	VII.	2.1×10^6	6.6×10^5
	X.	1.6×10^5	4.0×10^5
	mean	$\mathbf{6.2 \times 10^5}$	$\mathbf{5.8 \times 10^5}$

is made from pasteurized milk. Effective heat treatment leads to a minimization of the number of contaminating microbiota in milk.

Over four-year experiment, a series of microbiological analyses of farmer's cheese were carried out. In samples of cheese, there were determined the total counts of microorganisms, lactic acid bacteria, coliform bacteria, psychrotrophic microorganisms, enterococci and micromycetes (yeasts and moulds). The results of these analyses are presented in Tab. III and V.

Results of microbiological analyses of cheeses made by the farm A are listed in Tab. III. Determination of the TCM for foods containing cultural microflora is not given in the legislation because microbial culture, in our case mainly lactic acid bacteria, is dramatically reflected there. Therefore, TCM is not an indicator of increased microbial contamination there, but it provides a view of the number of microorganisms present in the product. TCM of the analysed cheeses reached high numbers – 10^5 to 10^9 CFU.g^{-1}. Average TCM for the entire period was 4.0×10^8 CFU.g^{-1} and a maximum TCM was 1.2×10^9 CFU.g^{-1}. LAB counts were usually lower than the TCM which was caused the most likely by the chosen methodology of the determination promoting the growth of some members of this diverse group. In fact, LAB is a group of bacteria with different demands on the cultivation temperature and atmospheric oxygen. Their numbers were in the range of several tens to 10^8 CFU.g^{-1}. The average value for the entire period was 3.8×10^7 and the maximum 2.8×10^8 CFU.g^{-1}. High initial density of LAB (10^6 – 10^7 CFU.g^{-1}) within the manufacture of fresh cheeses in conjunction with a suitable fermentation temperature (min. 18 °C) is able to keep undesirable microorganisms, such as e.g. *Staphylococcus aureus* and *E. coli*, under the control (Medveďová *et al.*, 2008).

In cheeses, there were repeatedly detected coliform bacteria exceeding the limit specified in ČSN 569609 (2.0 × 103 CFU.g^{-1}). The number of coliforms ranged from several bacteria up to 10^4 CFU.g^{-1}. Psychrotrophic microorganisms often reached high values, the maximum number was 1.0×10^6 CFU.g^{-1}. Enterococci counts were usually very low, only in exceptional cases the count exceeded 10^4 CFU.g^{-1}. Samples of cheese revealed the presence of micromycetes but their counts were

III: *Fresh goat cheese from conventional farm (A) – Statistical evaluation (descriptive statistics) of the whole monitored period*

Variable	Descriptive statistics (cheese produced at farm A)							
	N	Mean value	Median	Minimum	Maximum	Variance	Standard deviation	Coefficient of variation
TCM	40	4.02×10^8	3.20×10^8	9.09×10^5	1.22×10^9	9.3×10^{16}	3.06×10^8	76.03
LAB	40	3.83×10^7	9.00×10^6	50.0	2.80×10^8	4.9×10^{15}	7.03×10^7	183.27
Coli	40	9.96×10^3	1.73×10^3	5.0	1.16×10^5	4.1×10^8	2.03×10^4	203.76
Psychrotrophs	40	9.27×10^4	6.25×10^3	0.0	1.02×10^6	5.2×10^{10}	2.29×10^5	247.00
Enterococci	38	1.78×10^3	99	0.0	3.38×10^4	3.5×10^7	5.92×10^3	332.04
Micromycetes	38	6.93×10^3	10	0.0	2.57×10^5	1.7×10^9	4.16×10^4	600.78

Explanations: TCM – total counts of microorganisms, LAB – lactic acid bacteria, Coli – coliforms

IV: *Average counts and standard deviations of selected groups of microorganisms in fresh goat cheese produced on conventional farm A (CFU.g^{-1})*

Year	TCM		LAB		Coli		Psychrotrophs		Enterococci		Micromycetes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
I.	$7.2 \times 10^{8\,b}$	3.8×10^8	$5.0 \times 10^{4\,a}$	1.4×10^5	$2.9 \times 10^{3\,a}$	7.2×10^3	$2{,}0 \times 10^{2\,a}$	4.9×10^2	$7.1 \times 10^{3\,a}$	1.3×10^4	$6.5 \times 10^{2\,a}$	1.3×10^3
II.	$3.3 \times 10^{8\,a}$	3.0×10^8	$3.1 \times 10^{7\,a}$	8.1×10^7	$8.0 \times 10^{3\,a}$	1.4×10^4	$1.2 \times 10^{5\,a}$	2.9×10^5	$2.1 \times 10^{2\,a}$	4.6×10^2	$2.2 \times 10^{4\,a}$	7.4×10^4
III.	$3.4 \times 10^{8\,a}$	2.1×10^8	$7.7 \times 10^{7\,a}$	8.4×10^7	$1.7 \times 10^{4\,a}$	3.2×10^4	$1.1 \times 10^{5\,a}$	1.8×10^5	$1.4 \times 10^{3\,a}$	3.9×10^3	$6.8 \times 10^{1\,a}$	1.6×10^2
IV.	$2.7 \times 10^{8\,a}$	1.0×10^8	$3.1 \times 10^{7\,a}$	4.2×10^7	$9.0 \times 10^{3\,a}$	$1.3\text{x}10^4$	$1.2 \times 10^{5\,a}$	3.1×10^5	$7.3 \times 10^{2\,a}$	1.4×10^3	$1.1 \times 10^{1\,a}$	1.2×10^1

Explanations: TCM – total counts of microorganisms, LAB – lactic acid bacteria, Coli – coliforms, values marked with different letters indicate statistically significant difference ($p < 0.05$)

usually very low. Yeast dominated over moulds. Cheeses were not affected by moulds visibly.

The data in Tab. IV (conventional farm - A) show that statistically significant difference among individual years was not detected in the framework of selected groups of microorganisms, except TCM in the first year of monitoring. Therefore, cheeses were of relatively stable microbiological quality throughout the monitored period.

Results of microbiological analysis of fresh goat cheese produced on the farm B (organic farming) are indicated in Tab. V. Identical groups of microorganisms was analysed there. TCM ranged from 10^{7} to 10^{9} CFU.g^{-1}, the average number for the entire period was 6.9×10^{8} and a maximum 2.3×10^{9} CFU.g^{-1}. LAB counts ranged from several tens to 10^{8} CFU.g^{-1}, on average 1.6×10^{8} CFU.g^{-1}. The maximum count reached 9.2×10^{8} CFU.g^{-1}. Other groups of microorganisms varied in a wide range of several orders of magnitude. The maximum number of coliform bacteria was 2.4×10^{7} CFU.g^{-1} which is exceeding the limit specified by ČSN 569609 many times. Maximum count of psychrotrophic microorganisms was 2.2×10^{7} CFU.g^{-1}; enterococci 6.1×10^{6} CFU.g^{-1}, and micromycetes 2.4×10^{5} CFU.g^{-1}, again with a predominance of yeast.

The average values of selected groups of microorganisms for each year of the monitoring are indicated in Tab. VI. Unlike the previous farm (A), statistically significant difference in the framework of selected groups of microorganisms was found among individual years. Thus, cheese showed greater variability in microbiological quality.

Based on the comparison of the average numbers of the individual groups of microorganisms in cheeses from both farms (see Fig. 1), statistically significant difference was detected between the farms. Cheeses manufactured at the farm B compared with cheeses from the farm A were statistically different. Their microbiological quality was inferior. Higher numbers of coliforms, psychrotrophic microorganisms, enterococci and micromycetes were more frequently detected in the cheeses made by the farm B. Worse microbiological quality of the cheese was also reflected in their sensory properties, especially in the smell, colour and consistency, as it was evident within preparation of the individual samples. The worse microbiological quality of the cheeses

V: *Fresh goat cheese produced on organic farm B – statistical evaluation (descriptive statistics) during period monitored*

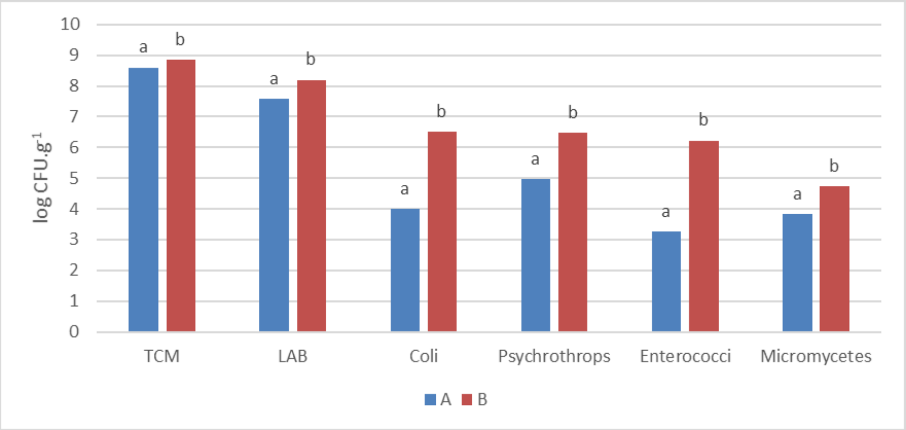
Variable	Descriptive statistics (cheese produced at farm B)							
	N	Mean	Median	Minimum	Maximum	Variance	Standard deviation	Coefficient of variation
TCM	33	6.95×10^{8}	3.40×10^{8}	4.00×10^{7}	2.39×10^{9}	4.5×10^{17}	6.72×10^{8}	96.67
LAB	33	$1.59 \text{ x } 10^{8}$	2.37×10^{7}	50	9.20×10^{8}	5.5×10^{16}	2.36×10^{8}	148.08
Coli	33	3.17×10^{6}	1.27×10^{6}	1.14×10^{2}	2.43×10^{7}	3.0×10^{13}	5.46×10^{6}	172.36
Psychrotrophs	33	3.03×10^{6}	3.45×10^{5}	5	2.24×10^{7}	2.5×10^{13}	5.04×10^{6}	166.23
Enterococci	31	1.78×10^{6}	4.68×10^{5}	3.34×10^{3}	$6.12\text{x } 10^{6}$	4.1×10^{12}	2.01×10^{6}	113.42
Micromycetes	31	5.48×10^{4}	9.00×10^{3}	0	2.39×10^{5}	6.6×10^{9}	8.10×10^{4}	147.84

Explanations: TCM – total counts of microorganisms, LAB – lactic acid bacteria, Coli – coliforms

VI: *Average counts and standard deviations of selected groups of microorganisms in fresh goat cheese produced on organic farm B (CFU.g-1)*

Year	TCM		LAB		Coli		Psychrotrophs		Enterococci		Micromycetes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
I.	$1.7\times10^{8\ \mathrm{a}}$	1.1×10^{8}	$1.5\times10^{4\ \mathrm{ab}}$	1.6×10^{4}	$5.1\times10^{3\ \mathrm{a}}$	6.7×10^{3}	$4.5\times10^{3\ \mathrm{a}}$	1.1×10^{4}	$1.2\times10^{4\ \mathrm{a}}$	1.2×10^{4}	$8.2\times10^{2\ \mathrm{a}}$	6.1×10^{2}
II.	$7.9\times10^{8\ \mathrm{ab}}$	7.9×10^{8}	$7.4\times10^{6\ \mathrm{a}}$	1.1×10^{7}	$1.0\times10^{6\ \mathrm{a}}$	1.2×10^{6}	$4.1\times10^{6\ \mathrm{ab}}$	4.5×10^{6}	$1.9\times10^{6\ \mathrm{ab}}$	2.0×10^{6}	$4.7\times10^{4\ \mathrm{a}}$	6.8×10^{4}
III.	$4.3\times10^{8\ \mathrm{ab}}$	1.8×10^{8}	$2.2\times10^{8\ \mathrm{bc}}$	1.6×10^{8}	$4.9\times10^{6\ \mathrm{a}}$	4.8×10^{6}	$6.6\times10^{6\ \mathrm{b}}$	7.3×10^{6}	$3.2\times10^{6\ \mathrm{b}}$	2.2×10^{6}	$9.2\times10^{4\ \mathrm{a}}$	9.4×10^{4}
IV.	$1.2\times10^{9\ \mathrm{b}}$	7.0×10^{8}	$4.2\times10^{8\ \mathrm{c}}$	3.0×10^{8}	$6.8\times10^{6\ \mathrm{a}}$	8.8×10^{6}	$1.9\times10^{5\ \mathrm{a}}$	1.8×10^{5}	$1.1\times10^{6\ \mathrm{ab}}$	1.5×10^{6}	$5.6\times10^{4\ \mathrm{a}}$	9.6×10^{4}

Explanations: TCM – total counts of microorganisms, LAB – lactic acid bacteria, Coli – coliforms, values marked with different letters indicate statistically significant difference ($p < 0.05$)



1: *Comparison of average counts of microorganisms in fresh goat cheese produced on conventional farm (A) and organic farm (B) for whole monitored period (CFU.g^{-1})*

Explanations: TCM – total counts of microorganisms, LAB – lactic acid bacteria, Coli – coliforms,
a,b – values marked with different letters indicate statistically significant difference ($p < 0.05$)

was probably due to the large farm reconstruction, including the production area. Drăghici *et al.* (2012) investigated distinction between organic and the conventional system of production of various foods. In samples of salty ewe cheese, following groups of microorganisms were determined: TCM, yeasts and fungi, *E. coli*/coliforms, *Enterobacteriaceae* and *Salmonella*. With respect to low counts of microorganisms, in the work of Draghici, no significant difference was found and in the framework of health safety, there is no significant difference between the groups.

As cheese is a dairy product, where lactic fermentation is a meaningful process, it is natural that TCM and LAB counts were high. Cheese produced on the farm A contained on average 4.0×10^8, resp. 3.8×10^7 CFU.g^{-1} (Tab. III). Cheese produced on farms B was higher on average in counts of both groups of microorganisms – 6.9×10^8, respectively 1.6×10^8 CFU.g^{-1} (Tab. V). There is clearly seen how significantly numbers of lactic acid bacteria are reflected in the TCM, even though LAB is a diverse group with different cultivation requirements. Similarly high numbers of LAB are presented in the study of Reis and Malcata (2011) investigating Portuguese cheeses made from goat and sheep milk.

In addition to the defined starter mesophilic cultures, undefined cultures obtained directly from milk, cheese, or previous processing participate in cheese manufacture. Non-starter lactic acid bacteria (NSLAB) are, as reported by Crow *et al.* (2001), a group consisting mainly of mesophilic lactobacilli (*Lactobacillus casei, L. rhamnosus, L. paracesei, L. plantarum, L. curvatus*) pediococci (*Pediococcus acidilactici, P. pentosaceus*) and enterococci (*Enterococcus feacalis, E. faecium*). This is confirmed by other studies, i.e. Kihal and Guessas (2004), Tserovska *et al.* (2002) and Tormo *et al.* (2015).

A possible source of NSLAB in cheese is raw milk, water, ingredients for the production of cheese, employees, production equipment, and air in a dairy plant (Madkor *et al.*, 2000; Crow *et al.*, 2001). Enterococci are an important group of NSLAB. In different goat cheeses, Cupáková *et al.* (2008) found relatively low numbers of these bacteria ranging from tens to 10^4 CFU.g^{-1}. During long-term monitoring, such numbers were found in cheeses produced at the farm A (Tab. III). Conversely, large numbers of enterococci from 10^4 to 10^7 CFU.g^{-1}, similar to counts found on the farm B (Tab. V), were detected by Franz *et al.* (2003), Foulquié Moreno *et al.* (2006) and De Fernando (2014). Martín-Platero *et al.* (2009) isolated 48 species of the genus *Enterococcus* in soft goat cheese, and 36 species in hard goat cheese. In goat smear-ripened cheese, there were identified *E. faecalis, E. faecium, E. durans, E. hirae* and *E. gallinarum* (Suzzi *et al.*, 2001). Enterococci were detected in 63.6% of samples of goat milk, specified as *E. faecalis* and *E. faecium* (Cortes *et al.*, 2006). According to these authors, enterococci can be the predominant microflora in cheese, as they may grow in environment high in salinity and low in pH. Their activities significantly contribute to the formation and maturation of aroma and taste. However, enterococci may also contribute to the formation of undesirable taste and biogenic amines in cheese (Greifová *et al.*, 2003; Hassan and Frank, 2014).

The major groups of microorganisms having an effect on the quality of cheese include psychrotrophic microorganisms. The most frequently isolated psychrotrophic bacteria from milk are representatives of the genera *Pseudomonas*, *Flavobacterium* and *Alcaligenes* (Burdová, 1998). Although representatives of other genera may be present in raw milk as well (Hassan and Frank, 2014; Nsofor and Frank, 2013). Some species of yeasts and moulds are also psychrotrophic. Thus, it is a very diverse group of microorganisms. Many psychrotrophic bacteria produce extracellular enzymes degrading proteins and lipids of milk. Defects caused by proteases are less common than defects caused by lipases, as proteases turn to pass into the whey while lipases remain trapped in the curd (Ducková and Čanigová, 2004). These microorganisms are ubiquitous and source of their contamination in milk can be water, soil, air, plants, animals and a man (Burdová, 1998). Our results (Tab. III and V) show cheeses contained relatively high numbers of psychrotrophic microorganisms, with some exceptions. For cheeses manufactured on the farm A, the average number for the entire period was 9.3 × 10^4 CFU.g^{-1} (Tab. II). On the other hand, cheeses made on the farm B were found many times higher in the average number of psychrotrophic microorganisms, amounted 3.0 × 10^6 CFU.g^{-1} (Tab. V). The relatively low numbers of psychrotrophic microorganisms in cheese from the farm A were reflected also in good sensory quality of the cheese, as large numbers of psychrotrophs can be associated with the development of sensory defects (taste, unpleasant odour) which could have been seen in the analysed cheeses from the farm B.

In milk, coliform bacteria are reliable indicator of primary and secondary contamination. Similarly, it indicates deficiencies in hygiene and sanitation within cheese production. Average counts of coliform bacteria in cheese from the farm A were 9.9 × 10^3 CFU.g^{-1}. At cheese from the farm B, much higher average count of coliforms 3.2 × 10^6 CFU.g^{-1} was found, see Fig. 1. Cupáková *et al.* (2008) detected considerably lower counts of coliform bacteria and *E. coli* (tens to 10^3 CFU.g^{-1}) in goat cheese purchased in the market of the South Moravian Region. Low numbers of *Enterobacteriaceae* were found in white cheeses by Šviráková *et al.* (2014). Reis and Malcata (2011) present considerable variability in the number of coliform bacteria in the range of 0 to 10^7 CFU.g^{-1} in Portuguese cheeses.

The main cause of the occurrence of contaminating yeasts and other microorganisms on the surface of the cheese is its excessive moisture. This can happen for more reasons (proteolysis, cheese “sweating”). Water and nutrients are accumulated between the surface of the cheese and its packaging material, and formed ideal environment for the growth of contaminating microorganisms (Johnson, 2001). Salt bath is a major source of contaminating yeast (Gőrner and Valík, 2004). There are most commonly isolated *Candida* spp., *Yarrowia lipolytica, Kluyveromyces marxianus, Geotrichum candidum, Debaryomyces hansenii* and *Pichia* spp. (Fleet, 1990; Hocking and Faedo, 1992; Rohm *et al.*, 1992; Lopandic *et al.* (2006); Fernandes, 2009). Reis and Malcata (2011) determined high yeast counts up to 10^7 CFU.g^{-1} in cheeses. Yeasts in goat milk were monitored by Fadda *et al.* (2010) who analysed samples of raw goat milk from 62 farms from different regions of Sardinia. *Candida zeylanoides* was determined as the most common type of yeast there.

Moulds tend to grow on cheeses where air bubbles between the packaging material and cheese are formed (Hocking and Faedo, 1992). Johnson (2001) reports *Penicillium* spp. is dominant fungi isolated from cheeses, though, he states *Aspergillus* spp. is dominating in the atmosphere of cheese processing plants. Our samples of cheeses accounted yeasts, as a commonly dominating group of micromycetes. High numbers of micromycetes, in particular yeasts exceeding the limits given by the references (i.e. The amount of 10^5 – 10^6 yeast per ml stated in Stratford (2006) detected in cheese from the farm B (Tab. V), were one of the causes of undesirable sensory changes (unpleasant taste and odour) at some of the cheeses.

The basic prerequisite for producing quality cheese is certainly quality milk. However, quality milk is not the only important factor. Equally important role is played by the proper technology and the consistent application of hygienic and sanitation measures. Any omission or failure to comply good practice can negatively affect the sensory characteristics of the cheese, without mentioning health safety. This might be cause of poor quality of cheese from the farm B. Although the farm B produces milk with appropriate microbiological quality, the sensory and microbiological quality of the cheese was worse, compared to the farm A.

CONCLUSION

The presented results draw the following conclusions. Within the monitored period, cheeses produced on the farm A exhibited relatively constant microbiological quality, unlike cheese produced on the farm B where statistically significant differences in the numbers of microorganisms were detected among the monitored years. Based on the comparison of the average numbers of individual groups of microorganisms in cheese, statistically significant difference between farms was determined. Cheeses produced on the farm B were of inferior microbiological quality. They were found to have high values of coliforms, enterococci, micromycetes and psychrotrophic microorganisms. Worse microbiological quality was also reflected in their sensory properties. The worse microbiological quality of the cheeses was probably due to the large farm reconstruction, including the production area. Microbiological and of course the qualitative parameters of cheeses produced at the farms are reflected in professional skills of the particular farmer, the technological advancement of farm operations, and compliance of hygienic-sanitation measures. For this reason, it is extremely important to continue intensively educating not only cheese producers but also the consumers.

Acknowledgements
This paper was supported by the projects of MSMT 2B08069 and NAZV QJ1210302

REFERENCES

BURDOVÁ, O. 1998. Quality milk products, depending on the microbial contamination of raw milk [in Slovak: Kvalita mliečnych výrobkov v závislosti od mikrobiálnej kontaminácie surového mlieka]. *Mliekarstvo*, 29(4): 44–45.

ČESKÝ NORMALIZAČNÍ INSTITUT. 2008. *Pravidla správné hygienické a výrobní praxe - Mikrobiologická kritéria pro potraviny. Principy stanovení a aplikace*. ČSN 56 9609 (569609) A. Praha: ČNI.

CORTES, C., DELAFUENTE, R., CONTRERAS, A., SANCHEZ, A., CORRALES, J., RUIZ SANTAQUITERIA, J. and ORDEN, J. 2006. Occurrence and preliminary study of antimicrobial resistance of enterococci isolated from dairy goats in Spain. *Int. J. of Food Microbiol.*, 110(1): 100–103.

CROW, V., CURRY, B. and HAYES, M. 2001. The ecology of non-starter lactic acid bacteria (NSLAB) and their use as adjuncts in New Zealand Cheddar. *Int. Dairy Journal*, 11: 275–283.

CUPÁKOVÁ, Š., NECIDOVÁ, L., DUŠKOVÁ, M., BELUŠÍKOVÁ, Z., JANŠTOVÁ, B., POSPÍŠILOVÁ, M. and KARPÍŠKOVÁ, R. 2008. Monitoring of microbiological guality of goats cheese from the retail market in the South Moravia [in Czech: Sledování mikrobiologické kvality kozích sýrů z tržní sítě Jihomoravského kraje]. In: *Celostátní přehlídky sýrů 2008. Výsledky přehlídek a sborník přednášek semináře Mléko a sýry*. Vysoká škola chemicko-technologická v Praze, January 23-24. Praha: Vysoká škola chemicko-technologická v Praze, pp. 123–126.

CUPÁKOVÁ, Š., POSPÍŠILOVÁ, M., LIPERTOVÁ, M., KOLÁŘOVÁ, I., KARPÍŠKOVÁ, R. and JANŠTOVÁ, B. 2006. Microbiological quality of goats milk [in Czech: Mikrobiologická kvalita kozího mléka]. In: *Celostátní přehlídky sýrů 2006. Výsledky přehlídek a sborník přednášek semináře Mléko a sýry.* Vysoká škola chemicko-technologická v Praze, January 25-26. Praha: Vysoká škola chemicko-technologická v Praze, pp. 59–63.

DE FERNANDO, G. G. 2014. *Enterococcus* in milk and Dairy Products. In: FUQUAY, J. W., FOX, P. F. AND MCSWEENEY, P. L. H. (EDS.). *Encyclopedia of dairy sciences. Vol. 3*. London: academic press, pp. 153–159.

DELGADO-PERTIÑEZ, M., ALCALDE, M. J., GUZMÁN-GUERRERO, J. L., CASTEL, J. M., MENA, Y. and CARAVACA, F. 2003. Effect of hygiene-sanitary management on goat milk quality in semi-extensive systems in Spain. *Small Ruminant Research*, 47: 51–61.

DRĂGHICI, M., NICULIŢĂ, P., GEICU, M., POPA A. AND DUŢA, D. 2012. ORGANIC VERSUS CONVENTIONAL FOOD PRODUCTS: PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSIS. *SCIENTIFIC BULLETIN, SERIES F, BIOTECHNOLOGIES*, 16: 149-155.

DRAGOUNOVÁ, H. and TOUŠOVÁ, R. 2008. *Practical Handbook for farm milk processing. Methodology for practice* [in Czech: *Praktická příručka pro faremní zpracování mléka. Metodika pro praxi*]. Praha: VÚM Praha, FAPPZ, ČZU v Praze.

DUCKOVÁ, V. and ČANIGOVÁ, M. 2004. Psychrotrophic microflora of milk [in Slovak: Psychrotrofná mikroflóra mlieka]. *Mliekarstvo*, 35(3): 32–35.

EL GALIOU, O., ZANTAR, S., BAKKALI, M., LAGLAOUI, A., CENTENO, J. A. and CARBALLO, J. 2015. Chemical and microbiological characteristics of traditional homemade fresh goat cheeses from Northern Morocco. *Small Ruminant Research*, 129: 108–113.

EUROPEAN PARLIAMENT. 2004. *Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs*. Brussels: The European Parliament and the Council of the European Union. Available at: http://data.europa.eu/eli/reg/2004/853/oj [Accessed: 2019, January 5].

EUROPEAN PARLIAMENT. 2007. *Commissionn Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs*. Brussels: The European Parliament and the Council of the European Union. Available at: https://eur-lex.europa.eu/eli/reg/2007/1441/oj [Accessed: 2019, January 5].

FADDA, M. E., VIALE, S., DEPLANO, M., PISANO, M. B. and COSENTINO, S. 2010. Characterization of yeast population and molecular fingerprinting of *Candida zeylanoides* isolated from goat's milk collected in Sardinia. *Int. J. of Food Microbiol.*, 136(3): 376–380.

FERNANDES, R. 2009. *Microbiology Handbook Dairy Products*. RSC Publ. and Leatherhead Food Int. Ltd. UK.

FLEET, G. H. 1990. Yeasts in dairy products. *J. Appl. Bacteriol.*, 68(3): 199–211.

FOULQUIÉ MORENO, M. R., SARANTINOPOULOS, P., TSAKALIDOU, E. and DE VUYST, L. 2006. The role and application of enterococci in food and health. *Int. J. of Food Microbiol.*, 106(1): 1–24.

FRANZ, C. M. A. P., STILES, M. E., SCHLEIFER, K. H. and HOLZAPFEL, W. H. 2003. Enterococci in foods – a conundrum for food safety. *Int. J. of Food Microbiol.*, 88(2–3): 105–122.

GÖRNER, F. and VALÍK, Ľ. 2004. *Applied food microbiology* [in Slovak: *Aplikovaná mikrobiológia požívatín*]. Bratislava: Malé centrum.

GREIFOVÁ, M., GREIF, G., LEŠKOVÁ, E. and MÉRIOVÁ, K. 2003. Enterococci - their evaluation in dairy technology [in Slovak: Enterokoky – ich hodnotenie v mliekarenskej technológii]. *Mliekarstvo*, 34(2): 42–45.

HASSAN, A. N. and FRANK, J. F. 2014. Microorganisms associated with milk. In: Fuquay, J. W., FOX, P. F. AND MCSWEENEY, P. L. H. (Eds.). *Encyclopedia of Dairy Sciences. Vol. 3.* London: Academic Press, pp. 447–457.

HOCKING, A. D. and FAEDO, M. 1992. Fungi causing thread mould spoilage of vacuum packaged cheddar cheese during maturation. *Int. J. of Food Microbiol.*, 16(2): 123–130.

JOHNSON, M. E. 2001. Cheese Products. In: MARTH, E. H. and STEELE, J. L. (Eds.). *Applied Dairy Microbiology*. New York: Marcel Dekker, Inc., pp. 345-384.

KIHAL, M. and GUESSAS, B. 2004. Characterization of lactic acid bacteria isolated from Algerian arid zone raw goats' milk. *African Journal of Biotechnology*, 3(6): 339–342.

LOPANDIC, K., ZELGER, S., BÁNSZKY, L. K., ELISKASES-LECHNER, F. and PRILLINGER, H. 2006. Identification of yeasts associated with milk products using traditional and molecular techniques. *Food Microbiol.*, 23(4): 341–350.

MADKOR, S. A., TONG, P. S. and SODA, M. 2000. Rippening of Cheddar cheese with added attenuated adjunct cultures of lactobacilli. *J. of Dairy Science*, 83(8): 1684–1691.

MARTÍN-PLATERO, A. M., VALDIVIA, E., MAQUEDA, M. and MARTÍNEZ-BUENO, M. 2009. Characterization and safety evaluation of enterococci isolated from Spanish goats' milk cheeses. *Int. J. of Food Microbiol.*, 132(1): 24–32.

MASOTTI, F., BATTELLI, G. and DE NONI, I. 2012. The evolution of chemical and microbiological properties of fresh goat milk cheese during its shelf life. *Journal of Dairy Science*, 95(9): 4760–4767.

MEDVEĎOVÁ, A., LIPTÁKOVÁ, D., VALÍK, Ľ., JANOVČÍKOVÁ, L. and HUDECOVÁ, A. 2008. Growth of microorganisms during the modelled manufacture of lump cheese produced from ewes and goat raw milk [in Slovak: Rast mikroorganismov počas modelových výrob hrudkových syrov zo surového ovčieho a kozieho mlieka]. In: *Celostátní přehlídky sýrů 2008. Výsledky přehlídek a sborník přednášek semináře Mléko a sýry*. Vysoká škola chemicko-technologická v Praze, January 23–24. Praha: Vysoká škola chemicko-technologická v Praze, pp. 131–136.

MORGAN, F., MASSOURAS, T., BARBOSA, M., ROSERIO, L., RAVASCO, F., KANDARAKIS, I., BONNIN, V., FISTAKORIS, M., ANIFANTAKIS, E., JAUBERT, G and RAYNAL-LJUTOVAC, K. 2003. Characteristics of goat milk collected from small and medium enterprises in Greece, Portugal and France. *Small Ruminant Research*, 47(1): 39–49.

NSOFOR, O. N. and FRANK, J. F. 2013. Milk and Dairy Products. In: DOYLE, M. P. AND BUCHANAN, R. L. (Eds.). *Food Microbiology: Fundamentals and Frontiers*. Washington, D. C.: ASM Press, pp. 169-185.

REIS, P. J. M. and MALCATA, F. X. 2011. Current state of Portuguese dairy products from ovine and caprine milks. *Small Ruminant Research*, 101(1-3): 122–133.

ROHM, H., ELISKASES-LECHNER, F. and BRÄUER, M. 1992. Diversity of yeasts in selelected dairy products. *J. of Appl. Bacteriol.*, 72(5): 370–376.

STRATFORD, M. 2006. Food and Beverage Spoilage Yeasts. In: QUEROL, A. and FLEET, G. H. (Eds.). *Yeasts in Food and Beverages*. Berlin/Heidelberg: Springer – Verlag, pp. 335–379.

SUZZI, G., CARUSO, M., GARDINI, F., LOMBARDI, A., VANNINI, L., GUERZONI, M. E., ANDRIGHETTO, C. and LANORTE, M. T. 2001. A survey of the enterococci isolated from an artisanal Italian goat's cheese (semicotto caprino). *J. of Applied Microbiol.*, 89(2): 267–274.

ŠVIRÁKOVÁ, E., CHRAMOSTOVÁ, J., MÜHLHANSOVÁ, A., PUKRTOVÁ, S., JEBAVÁ, I. and NĚMEČKOVÁ, I. 2014. Technologically risky microorganisms in white cheeses and salt brines [in Czech: Technologicky rizikové mikroorganismy v bílých sýrech a solných nálevech] . In: *Celostátní přehlídky sýrů 2014. Výsledky přehlídek a sborník přednášek semináře Mléko a sýry*. Vysoká škola chemicko-technologická v Praze, January 22–23. Praha: Vysoká škola chemicko-technologická v Praze, pp. 145–150.

TORMO, H., ALI HAIMOUD LEKHAL, D. and ROQUES, C. 2015. Phenotypic and genotypic characterization of lactic acid bacteria isolated from raw goat milk and effect of farming practices on the dominant species of lactic acid bacteria. *International J. of Food Microbiol.*, 210: 9–15.

TSEROVSKA, L., STEFANOVA, S. and YORDANOVA, T. 2002. Identification of lactic acid bacteria isolated from katyk, goat's milk and cheese. *J. of Culture Collections*, 3: 48–52.

Contact information

Libor Kalhotka: libor.kalhotka@mendelu.cz