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CHARACTERISTIC AND QUALITY AND FOOD SAFETY OF REGIONAL CHEESE PRODUCED FROM MIXED MILK

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Abstract

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There were cheeses produced from raw cow's milk and from mixed milk compared. Mixed milk contained small ruminants' milk (goat's and ewe's milk) and cow's milk in different proportions. There were technological, physical and health parameters, mineral composition, microbiological indicators and sensory quality evaluated. Cow's milk, compared to mixed milk, contained markedly lower amounts of fat, protein, casein, total solids, solids non fat, urea and acetone and higher values of lactose, citric acid and free fatty acids and showed significantly lower values of somatic cell count. Mixed milk showed lower (better) results for freezing point depression, markedly higher titration acidity and higher values for Ca, Mg, K, P, Cu, Mn and Zn. The results of microbiological analyses confirmed good hygienic quality in terms of total count of mesophlic, psychrotrophic and thermoresistant bacteria and coliforms. Negative incidence of *L. monocytogenes* and mostly negative incidence of *S. aureus* are important results and confirmed high quality of raw material for cheese production. None of *S. aureus* strains were confirmed as MRSA. The results of sensory evaluation showed no significant differences between cheeses originated from cow's milk and cheeses from mixed milk.

Keywords: cow's milk, goat's milk, ewe's milk, microbiology, technological parameters, macro-elements, micro-elements, senzory evaluation, *Listeria monocytogenes*, *Staphylococcus aureus*, MRSA

INTRODUCTION

Under the conditions of current economical climate, breeding of small ruminants and *in situ* processing of milk to farmer's regional products are still actual problems. Cheeses are produced and then mostly sold directly on farms or they are delivered into specialized stores with farmer's products in the Czech Republic. Products containing goat's and ewe's milk are popular mainly in persons with allergies to proteins of cow's milk or lactose intolerance. Main alergens of cow's milk are identified as casein (especially α-s1-casein) and whey proteins (α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulins). The prevalence of this allergic reaction can reach 26% in adults and up to 3% in children during the first three years (Bevilacqua *et al.*, 2000). Fortunately, more than 80% of children with allergy to milk recover (Host, 1998). Consumption of goat's milk confirmed the positive results (30–40%) in case of treated children with allergic reaction (Haenlein, 2004). The important characteristic of small ruminant's milk (goat's and ewe's milk) is also high content of short- and medium-chain fatty acids in milk fat that has at least twice more C6 – C10 fatty acids than cow's milk fat, namely 8%, 12% and 16% (cow's, ewe's and goat's milk fat, respectively; Chilliard *et al.*, 2006). Due to lower content of lactose, both goat's and ewe's milk and products are more suitable for people with lactose intolerance as a good source of calcium.

Food allergens can change during the heat or heatless processing into final products which can lead to changes in their allergenicity. The cow's milk which contains a complex of various properties of allergens (thermostable and thermolabile) can change the allergenic potential after pasteurization (60–70 °C for several seconds). So some individuals are allergic to cow's milk and they have an allergic reaction also after ingestion of cheese (Host and Samuelsson, 1988). One of the possibilities how to eliminate the alergical reaction and how to improve the human health is to exclude products from cow's milk from the diet or to reduce the occurrence of cow's milk in final products and replace cow's milk by goat's or ewe's milk. Because the consumption of goat's or ewe's milk products is not widespread in the Czech Republic (mostly due to the taste), there is a possibility to produce milk products from mixture of cow's and small ruminant's milk. Therefore, the importance of regional milk products is growing up.

Our study is focused on the development of cheese produced from cow's milk and mixed milk prepared from cow's, goat's and ewe's milk and on the evaluation and comparison of microbiological, technological and sensory characteristics of local specific product on one farm.

MATERIAL AND METHODS

Farm Characteristics

The farm is focused on the rearing of Holstein cows (H = 485), Cigája (Tsigai) sheep (C = 120) and White short haired goats (W = 80). The herds of cows and goats and flock of sheep are kept at an altitude of 432 m (H) and 474 m (C, W), total annual rainfall is around 800 (H) and 1200 mm (C, W) and average annual temperature is around 7.0 (H) and 3.7 °C (C, W). Dairy cows are grazed on pasture during year (summer) and animals are kept in free boxing stabling with mattresses. The cow milking is carried out in the carousel milking parlour (De-Laval), with eighteen sites. Goats and sheep are free during the vegetation period and through the day on natural pasture around the stable. The animals are milked twice a day in the milking parlour (Alfa-Laval) and are fed with a mixture of concentrates before milking. In the stable, they have access to hay, ad libitum.

Most of the areas used for grazing sheep and goats as well as cows, are permanent grassland of size 250–270 hectares for cows and 70–80 hectares for goats and sheep. There predominate the cultural mixtures suitable for Protected Landscape Area, such as meadow fescue (*Festuca pratensis*), timothy-grass (*Phleum pratense*) and cock's-foot (*Dactylis glomerata*), which are complemented by a mixture of red clover (*Trifolium pratense*) and white clover (*Trifolium repens*). The nutritional capacity of pastures varies (depends on the season) at the level of 40–60 kg per cow at all day pasture and 5–6 kg per one sheep or goat. In case of dairy cows in lactation, it is necessary to calculate the maximum 30 kg per animal and day. Pastures are used for 4–6 times per year, depending on the quality and nutritional capacity.

This farm has got a long years experience with production of mixture cheese from goat and ewe's sheep milk. Cheese is made as natural or flavored with different herbs and spices. Their speciality is cheese heated in hot whey which was twice awarded a prize „*Product of Olomouc region*“ (2007 and 2009).

Cheese Production Procedure

Raw cow's milk and raw mixed milk was precipitated enzymatically (diluted bacterial rennet Fromase 750 TL, France) in pasteurization double-walled stainless steel tank at temperature of 35 °C, with addition of $CaCl_2$. The time of precipitation usually lasted 20 minutes. Custard-like mass was cut into small pieces (15 × 15 cm) and then cut into smaller casein grain. The grain was mixed and inserted into forms for draining whey for 15 minutes. The forms with cheese were put in heated whey where they were pasteurized for 1.5–2 hours. Temperature of 85 °C must be kept in the bath at least for 1 hour. After this procedure the cheese was cooled, dried and manually salted by spreading NaCl over the surface for about 10 hours. The mixed milk was prepared in the proportions which are stated in the Tab. I.

Sample Collection

Throughout the monitoring period (May–August 2013) the following samples were collected during technology:

1) raw cow's milk and raw milk from small ruminants, which was mixed with cow's milk in different proportions which are shown in Tab. I;
2) whey from un-pasteurized milk after curdling;
3) whey after heating curd (85 °C pasteurization);
4) samples of final cheese (the surface and the inside of cheese were used for microbiological analyses).

Technological, Physical and Health Parameters

The following parameters were determined: milk fat (F; $g.100g^{-1}$; %), proteins (P; $g.100g^{-1}$; %), lactose (L; $g.100g^{-1}$; %), total solids (TS; $g.100g^{-1}$; %), solids non fat (SNF; $g.100g^{-1}$; %), casein (C; $g.100g^{-1}$; %), urea (U; $mg.100{\cdot}ml^{-1}$), citric acid concentration (CA; $mmol.l^{-1}$), free fatty acids content in fat (FFA; $mmol.100g^{-1}$), concentration of acetone (A; $mg.l^{-1}$), active acidity (pH), somatic cell count (SCC; $10^3.ml^{-1}$), inhibitory substances (IS), titration acidity (TA; in ml×2.5 $mmol.l^{-1}$ of NaOH solution), electrical conductivity (EC; $mS.cm^{-1}$), milk freezing point depression (FPD; °C), time of enzymatic coagulation (TEC; s), curd quality (CQ; from 1 = very good

I: *Proportions of milk for cheese production (in liter)*

Number of samples	date 2013	cow's milk	goat's milk	ewe's milk
A	14. 3.	120	-	-
B	23. 3.	120	-	-
1	14. 5.	120	5	80
2	12. 6.	50	20	30
3	12. 7.	80	78	50
4	19. 7.	80	78	52
5	25. 7.	80	78	52
6	21. 8.	100	60	40
7	23. 8.	100	70	30

to 4 = poor), curd firmness after enzymatic renneting (CF; cm – the less cm = the firmer curd); volume of whey curd cake (VW; ml), specific weight of milk and cheese (SW; $g.cm^{-3}$).

Fat in milk, cheese and whey was analyzed using Gerber method, lactose using polarimetric method. The reference method by Kjeldahl with Kjeltec Auto Distillation Unit 2200 (Foss-Tecator AB, Sweden; according to standard ČSN 57 0530) was used for determination of milk proteins in milk, cheese and whey, and for determination of casein in milk. In the case of milk MilkoScan 133B (Foss Electric, Denmark) infrared instrument was used for determination of F, P, L, SNF and TS content regularly with relevant biologically specific calibration (cow's, goat's and ewe's milk) as well. The composition of mixed milk was obtained after calculation of results of specific calibrations by weighted method according to milk proportions (Tab. I). Total solids and SNF in milk and whey were determined by drying in the hot air sterilizer (Chirana, Czech Republic) under conditions of the method and calculated after gravimetric measurement (Mettler, Switzerland) to obtain the results for the possibility of converting the total cheese composition (fat, protein and minerals) to values of total solids.

Milk urea, acetone and citric acid concentrations were determined by the spectrophotometric method (420, 485 and 428 nm) with p-dimethylaminobenzaldehyde (Ehrlich's reagent), KOH solution with salicylaldehyde and with pyridine in acetic anhydride using Spekol 11 device (Carl Zeiss Jena, Germany). Free fatty acids were measured on MIR-FT (Lactoscope FTIR, Delta Instruments, The Netherlands), pH at 20 °C on pH-meter CyberScan 510 (Eutech Instruments Europe B.V., Netherlands), electrical conductivity of milk at 20 °C on OK 102/1 conductometer (Radelkis, Hungary), titration acidity using method Soxhlet-Henkel according to ČSN 57 0530 standard, milk freezing point depression by cryoscopic method (Cryo-Star automatic, Funke-Gerber, Germany).

There were measured: time of enzymatic coagulation of milk protein; curd quality by subjective estimation using aspection and palpation, curd firmness after enzymatic renneting as a depth of penetration of the corpuscle falling into curd cake under constant conditions, whey volume of curd cake extracted from 50 ml of milk in the process of syneresis. Specific weight of milk and cheese was determined by gravimetric method. Inhibitory substances were analyzed by microbiological test (Delvo-test), somatic cell count by fluoro-opto-electronic method (Fossomatic 90, Foss Electric, Denmark).

Concentration of Macro- and Microelements in Cheese

Concentration of selected macroelements (Ca, Mg, Na, K, P) and microelements (Fe, Cu, Mn, Zn, Se) in the cheese was measured using atomic absorption spectrophotometry (AAS) on a device SOLLAR S4/M6 after mineralization of biological material. Mineralization of the sample (1 g) was performed using wet processs, and using nitric acid and hydrogen peroxide for 1 hour at 120 °C. To ensure the reliability of the results the samples mineralized during microwave heating were measured in parallel as well. There were 0.3 g of sample decomposed with nitric acid and hydrogen peroxide in three steps at temperatures of 145, 180 and 100 °C (for 5, 10 and 10 minutes). Final results were expressed as mean of all measurements.

Microbiological Analyses of Samples

The total count of mesophilic (TCM), psychrotrophic (TCP), and thermoresistant (TRM) microorganisms and coliforms (COLI), then *Staphylococcus aureus* (SA), methicilin resistant *S. aureus* (MRSA) and *Listeria monocytogenes* (LM) were determined in raw mixed milk, in whey from un-pasteurized milk after curdling and in heated whey and cheeses.

TCM were cultivated using GTK-M Agar at 30 °C/72 hours (according to standard ČSN EN ISO 4833), TCP were determinated using GTK-M Agar at 21 °C/25 hours (according to standard ČSN ISO 8552) and COLI on VČŽL Agar (Milcom, Tábor, CR) at 37 °C/24 hours by standard ČSN ISO 4832. The samples for enumeration of TRM were inactivated at 85 °C during 10 minutes and then cultivated on GTK Agar (Milcom, Tábor, CR) at 30 °C/72 hours (ČSN 57 0101).

Baird Parker Agar (HiMedia, India) was used for detection of *S. aureus*. Plates were incubated at 36 ± 1 °C for 48 h and black colonies with zones of precipitation were submitted to the tube for free coagulase test for confirmation. STAPHYtest and the identification program TNW Pro7.5 (Erba Lachema, s. r. o., Brno, Czech Republic) and BIOLOG III (Biolog, Ltd., Hayward, USA) were used for identification of isolated strains. The tentatively identified *S. aureus* isolates were confirmed by the multiplex PCR method for the detection of the species specific fragment SA442 (Martineau *et al*., 1998). MRSA and MR-CNS (methicillin resistant coagulase-negative staphylococci) were screened for the presence of *mecA* gene which encodes the resistance to methicillin (Boşgelmez-Tinaz *et al*., 2006).

CSN EN ISO 11290-1 standard was used for *L. monocytogenes* detection. Milk samples were enriched in half Fraser Broth (Oxoid, Basingstoke, UK) at 30 ± 1 °C for 24 h and then in Fraser Broth at 36 ± 1 °C for 48 h. This bacterial culture was then inoculated onto the surface of agar Oxford and PALCAM (Oxoid, Basingstoke, UK) and incubated at 36 ± 1 °C for 24 to 48 h. Colonies suspected to be *L. monocytogenes* were confirmed by the catalase test. Then, catalase positive colonies were inoculated onto the Rapid'L Mono Agar and AL (Lot) Agar *Listeria* (Bio-Rad, Laboratories, Inc., US) and incubated at 36 ± 1 °C for 24 h. All suspected organisms were sent for confirmation to the Reference laboratory for bacteria genus *Listeria* (National Institute of Public Health, Centre of Food Chain Hygiene, Brno).

Senzory Evaluation

In sensory evaluation were assessed: visual aspect (appearance), consistency (intensity of hardness), odour, taste, taste for ewe's/goat's milk, the overall impression. The laic public, composed of 4 male and 4 female population of different ages, participated in assessment. Cheeses were graded from 1 to 5, where 1 was the best mark.

RESULTS AND DISCUSSION

Technological, Physical and Health Parameters of Raw Milk

Evaluation of the composition and properties of a pool of raw milk as a raw material for the production of cheese produced some interesting results (Tabs. II, III, IV and V). Cow's milk, compared to mixed milk but at different stages of the season (cow's milk in early spring, milk mixed in spring and summer), contained markedly lower amounts of fat (3.70 for cow's milk and 3.99 for mixed milk), protein (3.09 and 3.52), casein (2.14

II: *The basic composition of the cow's bulk milk*

Parameter	F	P	L	SNF	TS	C	U	CA	FFA	A
x	3.70	3.09	4.8	6.58	12.22	2.14	20.4	8.17	0.885	4.84
sd	0.113	0.057	0.113	2.8	1.212	0.099	1.79	0.013	0.007	0.643
min	3.48	2.98	4.58	10.9	11.8	1.95	16.89	8.14	0.871	3.58
max	3.92	3.2	5.02	12.1	12.64	2.33	23.91	8.2	0.899	6.1

x = arithmetic mean; sd = standard deviation; min – max = 95% confidence interval of value incidence calculated as 1.96 x sd ± x; F = milk fat; P = proteins; L = lactose; SNF = solids non fat; TS = total solids; C = casein; U = urea; CA = citric acid; FFA = free fatty acids; A = acetone

III: *The basic composition of the mixed milk*

Parameter	F	P	L	SNF	TS	C	U	CA	FFA	A
x	3.99	3.52	4.7	8.89	12.92	2.65	37.48	8.04	0.588	6.28
sd	0.475	0.303	0.061	0.316	0.884	0.247	2.97	0.031	0.283	0.06
min	3.06	2.93	4.58	8.27	11.18	2.17	31.65	7.98	0.033	6.16
max	4.92	4.11	4.81	9.51	14.65	3.13	43.3	8.1	1.143	6.4

IV: *Health, physical and technological indicators of cow's milk*

Parameter	SCC	FPD	SW	pH	EC	TA	TEC	CQ	CF	VW
x	269	–0.5181	1.0311	7.03	3.35	7.4	154.5	3	1.75	29.5
sd	39.6	0.002	0.002	0.035	0.042	0.225	19.1	0	0.071	0.71
min	191	–0.522	1.0272	6.96	3.27	6.96	117.1	-	1.61	28.1
max	347	–0.5142	1.035	7.1	3.43	7.84	191.9	-	1.89	30.9

x = arithmetic mean; sd = standard deviation; min – max = 95% confidence interval of value incidence calculated as 1.96 x sd ± x; SCC = somatic cell count; FPD = freezing point depression; SW = specific weight; pH = active acidity; EC = electrical conductivity; TA = titration acidity; TEC = time of enzymatic coagulation; CQ = curd quality; CF = curd firmness; VW = volume of whey

V: *Health, physical and technological indicators of mixed milk*

Parameter	SCC	FPD	SW	pH	EC	TA	TEC	CQ	CF	VW
x	643	–0.5365	1.0371	6.98	4.22	8.66	98	2	1.7	32
sd	219.4	0.008	0.001	0.042	0.473	0.922	25.5	1.22	0.264	3.02
min	213	–0.5522	1.0351	6.9	3.29	6.85	48	1 (k)	1.18	26.1
max	1073	–0.5208	1.0391	7.06	5.15	10.47	148	4 (k)	2.22	37.9

and 2.65), total solids (12.22 and 12.92), solids non fat (6.58 and 8.89), urea (20.40 and 37.48) and acetone (4.84 and 6.28). On the contrary, this milk had higher values of lactose (4.80 and 4.70) and free fatty acids (0.885 and 0.588). The differences were generally in accordance with the previously mentioned differences between cow's milk and milk of small ruminants (Genčurová *et al.*, 2008; Zoa-Mboé *et al.*, 1997). These differences confirm a higher technology (cheese technology) recovery for mixed milk, e.g. casein (Tabs. II and III). Higher content of urea is also typical for small ruminant's milk. The content of free fatty acids in both types of milk indicates a good quality and healthy animals. All values are under FFA qualitative limits specified by ČSN 57 0529 standard.

As expected, the larger difference was observed in somatic cell count. Cow's milk showed significantly lower values (Tabs. IV and V), which are typical in comparison to milk of small ruminants(269,000 vs. 643,000 cells/ml). Souza *et al.* (2009) mentioned the SCC average 779,000 cells/ml in 13 goat herds. Hanuš *et al.* (2009) detected the 4,267,400 cells/ml in one goat herd (White short haired), 948,500 cells/ml in one sheep flock (Tsigai) and 230,000 cells/ml in three dairy cow herds with Czech Fleckvieh cattle. Fekadu *et al.* (2004) mentioned the average 1,905,461 SCC/ml in bulk milk in Alpine goat herd. Paape *et al.* (2007) found that SCC increased with increasing of parity and stage of lactation for goats and cows but not for sheep. For goats, by the fifth parity the SCC increased from 250,000 SCC/ml after parity to 1,150,000/ml at 285 days of lactation. Adwan *et al.* (2005) compared the SCC between milk from healthy animals and animals with bacterial mastitis infection (cows, goats and sheep). They found significantly higher SCC from infected animals: 520,000 vs. 140,000 cells/ml (cows), 1,650,000 vs. 490,000 cells/ml (goats) and 1,420,000 vs. 330,000 (sheep). The standard limit value for SCC is not determined in our country (also in EU countries) as is determined in US (SCC < 1,000,000 in 1 ml). In general, SCC of raw materials applied in our study was good for food production in terms of health and physiological context (with respect to udder health).

The freezing point depression was lower (better) for mixed milk (–0.5365 compared to cow's –0.5181) due to its higher content of solid components, total solids, solids non-fat, urea, organic and inorganic salts (demonstrated by significantly higher electrical conductivity) as its lower lactose content could not cause this effect (Tabs. IV and V). For the same reasons, mixed milk had also slightly higher specific weight (1.0371) as compared to cow's milk (1.0311), while the pH was similar in terms of buffering capacity (6.98 mixed milk, 7.03 cow's milk). The similar results described in the previous work Genčurová *et al.* (2008) and Macek *et al.* (2008) for the same breeds of goats and sheep.

In part due to higher content of protein and casein, the mixed milk has markedly higher titration acidity 8.66 °SH (cow's milk 7.40). Cheese-making technological properties (shorter time of coagulation – 98 versus 154.5 s), similar curd firmness (1.7 vs. 1.75 cm) and larger volume of whey (32 vs. 29.5 ml) appeared as expected favorable for milk mixed (Tabs. IV and V).

The inhibitory substances in different types of milk were not detected by the method used. These results confirm also a good control technology in animal breeding and hygiene rules during the milking process.

Basic Parameters of Whey

The obtained results follow the technological changes during the manufacturing process and value min to max allow estimation of the final condition sub-raw materials (Tabs. VI–IX). The whey after coagulation of cow's milk is in its basic composition very similar to the whey of mixed milk with a higher fat (see Tabs. VI and VII), of which capture in the whey is very variable with respect to the course of syneresis process. The ratios may be also well reversed. Slightly less protein amount was detected in whey from cow's milk (0.83 vs. 0.98; Tabs. VI and VII) which corresponds to differences in the initial (default) raw materials with a lower protein content of cow's milk versus the mixed milk (3.09 vs. 3.52; Tabs. II and III). Significantly lower variability of proteins compared to fat ($F = 1.31$ cow's milk vs. 1.05 mixed milk; Tabs. VI and VII) is interesting in both whey as proof of the effectiveness and impact of the enzymatic coagulation when the influence to proteins is direct (biochemical), while indirect to fat (hydromechanical). It means that the repeatability of syneresis process in terms of fat yield is not well balanced, which is affected by many factors such as health and animal nutrition. Average values of lactose (5.24 cow's milk vs. 5.18 mixed milk) and solids non fat (6.71 vs. 6.80), and their variability are very similar in both of wheys (see Tabs. VI and VII).

VI: *The basic composition of whey after coagulation of cow's milk in the cheese production technology*

Parameter	F	P	L	SNF
x	1.31	0.83	5.24	6.71
sd	0.255	0.014	0.156	0.24
min	0.81	0.8	4.93	6.24
max	1.81	0.86	5.55	7.18

x = arithmetic mean; sd = standard deviation; min – max = 95% confidence interval of value incidence calculated as 1.96 x sd ± x; F = milk fat; P = proteins; L = lactose; SNF = solids non fat

VII: *The basic composition of whey after coagulation of mixed milk in the cheese production technology*

Parameter	F	P	L	SNF
x	1.05	0.98	5.18	6.8
sd	0.58	0.062	0.122	0.115
min	0.47 (k)	0.86	4.94	6.57
max	1.63 (k)	1.10	5.42	7.03

x = arithmetic mean; sd = standard deviation; min – max = 95% confidence interval of value incidence calculated as 1.96 x sd ± x; F = milk fat; P = proteins; L = lactose; SNF = solids non fat; k = corrected calculation (taking account of qualified estimate for large dispersion of basic data)

VIII: *The basic composition of heated whey (85 °C) from cow's milk in the cheese production technology*

Parameter	F	P	L	SNF
x	0.26	0.69	5.38	6.68
sd	0.021	0.021	0.141	0.219
min	0.22	0.65	5.10	6.25
max	0.3	0.73	5.66	7.11

IX: *The basic composition of heated whey (85 °C) from mixed milk in the cheese production technology*

Parameter	F	P	L	SNF
x	0.5	0.6	5.41	6.62
sd	0.469	0.12	0.128	0.241
min	0.031 (k)	0.36	5.16	6.15
max	0.969 (k)	0.84	5.66	7.09

Basic Parameters of Heated Whey (at 85 °C)

The results recorded differences in the types of milk and changes in the technological process. The results indicate decreasing of fat (0.26 cow's milk and 0.50 mixed milk) and protein percentage (0.69 cow's milk and 0.60 mixed milk) in heated whey as compared to whey after coagulation (Tabs. VIII and IX versus VI and VII). Evaporation should theoretically increase the concentration values, but due to the subsequent thermal precipitation of proteins and fat globules capturing in newly emerging structures and due to their mechanical collecting during processing the mentioned decreases are resulting (Tabs. VI–IX), which may be around 20 to 75%. Conversely, as shown and previously stated, the lactose was classically concentrated by heating and water steam evaporation in both of whey (5.38 cow's milk and 5.41 mixed milk). Due to the above contradictory trends (bilateral slips) the consequence is that solids non fat content in the whey is not only similar between the types of milk (Tab. VI and VII) but also after heating process (6.68 cow's milk vs. 6.62 mixed milk; Tabs. VIII and IX).

Basic Chemical Composition of Cheese

The basic chemical composition of cheese produced from cow's and mixed milk is shown in Tabs. X and XI and provides important dietetic information. The nutritional values of cheese made from raw materials of both types (cow's and mixed milk) typically show the higher variation (with certain exceptions which are specified by chance rather a lower frequency of samples, e.g. cheese from cow's milk), which is usually typical for processing of smaller quantities of raw milk depending on the season, but it does not affect product quality in essential way.

Mineral Composition of Cheese

The concentration of macro- and microelements in evaluated cheeses (from cow's and mixed milk) in the original matter and dry matter are shown in Tabs. XII to XV. The results represent

X: *Basic chemical compositoin of cheese produced from cow's milk*

Parameter		Unit
milk fat	27.9 ± 3.39	%
proteins	20.29 ± 1.46	%
total solids	54.89 ± 6.59	%
fat in total solids	50.83 ± 0.09	%
proteins in total solids	37.06 ± 1.78	%
specific weight	1.0318 ± 0.007	$g.cm^{-3}$

XI: *Basic chemical compositoin of cheese produced from mixed milk*

Parameter		Unit
milk fat	30.32 ± 4.13	%
proteins	22.15 ± 1.65	%
total solids	55.1 ± 1.96	%
fat in total solids	54.95 ± 6.68	%
proteins in total solids	40.21 ± 2.68	%
specific weight	1.0907 ± 0.055	$g.cm^{-3}$

XII: *Macroelements composition in cheese (in original mass of cheese) from cow's milk and mixed milk*

Macroelement		Ca	Mg	Na	K	P
		mg.kg–1				
cow's milk	x	7,649	364	280	802	4,865
	sd	466.1	35.8	32.7	74.7	546
	min	6,735	294	216	656	3,795
	max	8,563	434	344	948	5,935
mixed milk	x	8,966	449	248	810	5,416
	sd	796.1	26.5	20.4	54.4	440.3
	min	7,406	397	208	703	4,598
	max	10,526	501	288	917	6,279

x = arithmetic mean; sd = standard deviation; min – max = 95% confidence interval of value incidence calculated as 1.96 x sd ± x; Ca = calcium; Mg = magnesium; Na = natrium; K = kalium; P = phosphorus

XIII: *Microelements composition in cheese (in original mass of cheese) from cow's milk and mixed milk*

Microelement		Fe	Cu	Mn	Zn	Se
		$mg.kg^{-1}$				
cow's milk	x	5.58	0.7	0.5	34.9	0.1
	sd	1.91	0.174	0.131	4.29	0.006
	min	1.84	0.36	0.24	26.5	0.1
	max	9.32	1.04	0.76	43.3	0.12
mixed milk	x	5.36	1.1	0.62	35.1	0.08
	sd	1.17	0.243	0.207	2.71	0.015
	min	3.07	0.62	0.21	29.8	0.05
	max	7.65	1.58	1.03	40.4	0.11

Fe = ferrum; Cu = cuprum; Mn = manganum; Zn = zinc; Se = selenium

the expected average values and their variability under the influence:

- probable mixing ratio of raw material with regard to the biological species milk;
- season and related nutrition;
- stage of lactation;
- specific animal health;
- breeds.

The higher values for the mixed milk were measured for Ca 8,966 $mg.kg^{-1}$, Mg 449 $mg.kg^{-1}$, K 810 $mg.kg^{-1}$, P 5,416 $mg.kg^{-1}$, Cu 1.1 $mg.kg^{-1}$, Mn 0.62 $mg.kg^{-1}$, Zn 35.1 $mg.kg^{-1}$, and that in the original

XIV: *Macroelements composition in cheese (in dry matter) from cow's milk and mixed milk*

Macroelement		Ca	Mg	Na	K	P
		mg.kg⁻¹				
cow's milk	x	1,394	66	51	146	886
	sd	84.9	6.5	6	13.6	99.5
	min	1,351	53	39	119	691
	max	1,437	79	63	173	1,081
mixed milk	x	1,627	81	45	147	983
	sd	144.5	4.8	3.7	9.9	79.9
	min	1,344	72	38	128	826
	max	1,910	90	52	166	1,140

XV: *Microelements composition in cheese (in dry matter) from cow's milk and mixed milk*

microelement		Fe	Cu	Mn	Zn	Se
		mg.kg⁻¹				
cow's milk	x	101.7	12.8	9.11	634.9	2.004
	sd	34.83	3.17	2.387	78.12	0.109
	min	33.4	6.6	4.3	481.8	1.79
	max	170.0	19.0	13.79	788.0	2.218
mixed milk	x	97.3	20.0	11.25	636.1	1.452
	sd	21.31	4.41	3.757	49.26	0.272
	min	55.5	11.4	3.89	539.6	0.919
	max	139.1	28.6	18.61	732.6	1.985

mass of cheese. In case of dry matter, there were these values in the mixed milk for Ca 1,627 mg.kg^{-1}, Mg 81 mg.kg^{-1}, K 147 mg.kg^{-1}, P 983 mg.kg^{-1}, Cu 20.0 mg.kg^{-1}, Mn 11.25 mg.kg^{-1}, Zn 636.1 mg.kg^{-1}. These results correspond with the content of elements in raw milk of small ruminant (Hanuš *et al.*, 2008).

Evaluation of Selected Microbiological Indicators

Cooled bulk cow's milk showed a good hygienic quality (TCM 10,714, TCP 8,143, TRM 58 and COLI 53 CFU.ml^{-1}) corresponding to CSN 570529 and Regulation (EC) No 853/2004 (Tab. XVI). Milk of small ruminants is not directly introduced in the Czech Republic hygiene regulations. There is only stated that the milk of other animals (other then cows) can not exceed the limit for TCM 1,500,000 CFU.ml^{-1} (Regulation EC 853/2004). Experience of similar works show generally higher values of hygiene indicators (SCC, but also TCM) since used programs of mammary gland toilet are mostly not as well-ordered as by the cow's milk production (Muehlherr *et al.*, 2003; Gonzalo *et al.*, 2006). This was confirmed also by our results (TCM 74,909, TCP 16,291, TRM 72 and COLI 2,345 CFU.ml^{-1}). With regard to the evaluation of cheeses, the microbiological contamination was as expected worse on the surface than inside the cheeses (Tab. XVI).

Negative incidence of *L. monocytogenes* and mostly negative incidence of *S. aureus* is an important result and confirms a high quality of raw material for cheese production. The higher occurrence of *S. aureus* in raw mixed milk and in whey after coagulation is probably due to contamination of cow's milk, which confirms our earlier results, where we stated higher incidence of *S. aureus* in cow's milk compared to milk of small ruminants – 45% (cow's), 17% (goat's) and 25% (ewe's) – Vyletělová *et al.* (2011a). Oprean *et al.* (2011) presented the similar results. They found *S. aureus* occurrence in cow's, goat's and ewe's milk 70%, 20% and 20%, respectively. No *S. aureus* was confirmed as methicilin resistant strain (MRSA) opposed to our earlier study (Vyletělová *et al.*, 2011b). There we confirmed the MRSA occurrence mainly in cow's individual milk (6.1% of strains identified as *S. aureus*), in goat's milk (5.0%) and in ewe's milk the occurrence of MRSA was negative.

Evaluation of Sensory Characteristics of Cheese

The results of sensory evaluation showed that there were no significant differences between cheeses originated from cow's milk and cheeses from mixed milk. The final results of assessed parameters are summarized in Tab. XVII.

Appearance – there were found values 2.21 ± 0.11 for cheese from cow's and values 2.55 ± 0.43 for cheese from mixed milk. These results were similar and they can be described as good even very good. **Consistency** – there were found minimal differences. The value 3.79 ± 0.3 was determined for cow's milk and 3.89 ± 0.24 for mixed milk. **Odour**

XVI: *Results of microbiological analyses*

Parameter	CFU.ml^{-1}	TCM	TCP	TRM	COLI	SA	LM
	raw milk						
cow's milk	x	18,000	13,667	60	1,005	11	neg.
	g	10,714	8,143	58	53	3	neg.
mixed milk	x	109,000	29,520	91	4,000	4,942	neg.
	g	74,909	16,291	72	2,345	1,490	neg.
	whey after coagulation						
cow's milk	x	40,000	14,000	16	1,650	neg.	neg.
	g	35,496	8,660	15	949	neg.	neg.
mixed milk	x	179,429	41,086	31	28,190	578	neg.
	g	45,632	4,427	22	3,236	19	neg.
	heated whey						
cow's milk	x	1,027	16	15	8	neg.	neg.
	g	989	15	15	7	neg.	neg.
mixed milk	x	222	50	8	4	neg.	neg.
	g	63	25	5	2	neg.	neg.
	cheese surface						
cow's milk	x	250,000	84,005	310	33,000	neg.	neg.
	g	150,000	1,296	110	32,863	neg.	neg.
mixed milk	x	4,511,513	1,015,678	53	259,513	neg.	neg.
	g	28,794	995	18	206	neg.	neg.
	cheese inside						
cow's milk	x	182,000	62,505	230	8,275	neg.	neg.
	g	70,000	1,118	67	2,019	neg.	neg.
mixed milk	x	1,204,474	430,994	45	45,731	neg.	neg.
	g	10,234	1,154	12	90	neg.	neg.

x = arithmetic mean; g = geomean; TCM = total count of mesophilic microorganisms; TCP = total count of psychrotrophic microorganisms; TRM = thermoresistant microorganisms; COLI = coliforms; SA = *Staphylococcus aureus*; LM = *Listeria monocytogenes*

XVII: *Results of sensory evaluation*

Milk	appearance	consistency	odour	taste	taste for sheep/goat	overall impression
cow's	2.21 ± 0.11	3.79 ± 0.30	3.07 ± 0.91	3.15 ± 1.21	1.15 ± 0.21	2.86 ± 1.21
mixed	2.55 ± 0.43	3.89 ± 0.24	2.50 ± 0.44	2.71 ± 0.46	1.61 ± 0.63	2.62 ± 0.52

– there were determined values 3.07 ± 0.91 for cow's and 2.5 ± 0.44 for mixed milk. There was a relatively large difference, which can be interpreted as good to very good, which also does not show any expected worse odour of cheese from the milk of small ruminants. **Taste** – in this important category, there were values of 3.15 ± 1.21 for cow's cheese and better values 2.71 ± 0.46 for cheese from mixed milk. This difference can be explained by the presence of the proportion of sheep's milk (traditionally predetermined for cheese production) and higher fat content. **Taste for goat's or sheep milk** – in this specific category were detected values of 1.15 ± 0.21 (cow's milk) and 1.61 ± 0.63 (mixed milk) which was expected result. This result indicates a good pasture and hygienic conditions in animal breeding. These findings open the possibility for higher consumption, which could be lower in case of stronger taste. **Overall impresion** – in this category the results were similar 2.86 ± 1.21 and 2.62 ± 0.52, which is also for sale of cheeses from milk mixed important.

CONCLUSION

There were compared technological, physical and health parameters, and then mineral composition, microbiological indicators and sensory evaluation between cheeses produced from cow's milk and from mixed milk (cow's, goat's and ewe's). The following conclusions are evident from the results:

1) cow's milk, compared to mixed milk, contained markedly lower amounts of fat, protein, casein, total solids, solids non fat, urea and acetone. On the contrary, this milk had higher values of lactose and free fatty acids;

2) cow's milk showed significantly lower values of somatic cell count;
3) the freezing point depression was lower (better) for mixed milk;
4) the mixed milk showed markedly higher titration acidity, higher content of protein and casein, higher content of solid component, total solids, solids non-fat, urea, organic and inorganic salts;
5) cheese-making technological properties (shorter time of coagulation), similar curd firmness and larger volume of whey appeared, as expected, favorable for mixed milk. Inhibitory substances were negative in all of samples;
6) mineral composition in cheese shows the higher values for the mixed milk for Ca, Mg, K, P, Cu, Mn;
7) bulk cow's milk showed a good quality in terms of TCM, TCP, TRM and COLI. Milk of small ruminants generally has the higher values of microbiological indicators. Negative incidence of *L. monocytogenes* and mostly negative incidence of *S. aureus* is an important result and confirms high quality of raw material for cheese production;
8) the results of sensory evaluation showed that there were no significant differences between cheeses originated from cow's milk and cheeses from mixed milk. This result indicates a good pasture and hygienic conditions in animal breeding.

SUMMARY

In regional cheese development there were determined the following parameters: milk fat (F; $g.100g^{-1}$; %), proteins (P; $g.100g^{-1}$; %), casein (C; $g.100g^{-1}$; %), lactose (L; $g.100g^{-1}$; %), total solids (TS; $g.100g^{-1}$; %), solids non fat (SNF; $g.100g^{-1}$; %), urea (U; $mg.100{\cdot}ml^{-1}$), citric acid concentration (CA; $mmol.l^{-1}$), free fatty acids content in fat (FFA; $mmol.100g^{-1}$), concentration of acetone (A; $mg.l^{-1}$), active acidity (pH), somatic cell count (SCC; $10^3.ml^{-1}$), inhibitory substances (IS), titration acidity (TA; in ml×2.5 $mmol.l^{-1}$ of NaOH solution), electrical conductivity (EC; $mS.cm^{-1}$), milk freezing point depression (FPD; °C), time of enzymatic coagulation (TEC; s), curd quality (CQ; from 1 = very good to 4 = poor), curd firmness after enzymatic renneting (CF; cm – the less cm = the firmer curd); volume of whey curd cake (VW; ml), specific weight of milk and cheese (SW; $g.cm^{-3}$), concentration of selected macroelements (Ca, Mg, Na, K, L) and microelements (Fe, Cu, Mn, Zn, Se) in the cheese ($mg.kg^{-1}$), total count of *Staphylococcus aureus* (SA), methicilin resistant *S. aureus* (MRSA), *Listeria monocytogenes* (LM), mesopihilic (TCM), psychrotrophic (TCP) and thermoresistant (TRM) microorganisms and coliforms (COLI) were determined in raw mixed milk, unpasteurized and heated whey and cheese (in $CFU.ml^{-1}$) and sensory characteristics (visual aspect (appearance), consistency (intensity of hardness), odour, taste, taste of ewe's/goat's milk, the overall impression) which were evaluated by the laic public, composed of male and female population of different ages, participated in assessment. Cheeses were graded from 1 to 5, where 1 was the best mark. From our results is evident that cow's milk, compared to mixed milk contained markedly lower amounts of fat (3.70 cow's milk and 3.99 mixed milk), protein (3.09 and 3.52), casein (2.14 and 2.65), total solids (12.22 and 12.92), solids non fat (6.58 and 8.89), urea (20.4 and 37.48) and acetone (4.84 and 6.28), somatic cell count (269,000 and 643,000 cells/ml), titration acidity (7.4 and 8.66). On the contrary, this milk had higher values of lactose (4.80 and 4.70) and free fatty acids (0.885 and 0.588). The freezing point depression was lower (better) for mixed milk (–0.5365 and –0.5181) and inhibitory substances were negative in all of samples. Cheese-making technological properties (shorter time of coagulation – 98 versus 154.5), similar curd firmness (1.7 vs. 1.75) and larger volume of whey (32 vs. 29.5) appeared as expected favorable for mixed milk. Mineral composition in cheese shows the higher values for the mixed milk for Ca 8,966 $mg.kg^{-1}$, Mg 449 $mg.kg^{-1}$, K 810 $mg.kg^{-1}$, P 5, 416 $mg.kg^{-1}$, Cu 1.1 $mg.kg^{-1}$, Mn 0.62 $mg.kg^{-1}$, Zn 35.1 $mg.kg^{-1}$ in the original mass of cheese and values for Ca 1,627 $mg.kg^{-1}$, Mg 81 $mg.kg^{-1}$, K 147 $mg.kg^{-1}$, P 983 $mg.kg^{-1}$, Cu 20.0 $mg.kg^{-1}$, Mn 11.25 $mg.kg^{-1}$, Zn 636.1 $mg.kg^{-1}$ in dry matter. Bulk cow's milk confirmed the good hygienic quality of this farm in terms of TCM 10,714, TCP 8,143, TRM 58 and COLI 53 $CFU.ml^{-1}$. Milk from small ruminants had expected higher values – TCM 74,909, TCP 16,291, TRM 72 and COLI 2,345 $CFU.ml^{-1}$. Negative incidence of *L. monocytogenes* and mostly negative incidence of *S. aureus* is an important result and confirms a high quality of raw material for cheese production. The results of sensory evaluation showed that there were no registered significant differences between cheeses originated from cow's milk and cheeses from mixed milk. This result indicates a good pasture and hygienic conditions in animal breeding.

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