Microorganisms and volatile aroma-active compounds in “nite“ and “vojky” cheeses

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Summary

“Nite“ and “vojky“ are various string-like forms of a traditional cheese in Slovakia. These products are manufactured by shaping of pasteurized or raw cows’ or ewes’ milk-based lump cheeses after melting in hot water. Unsmoked versions of these cheeses from three producers were studied regarding the contents of microorganisms and volatile aroma-active compounds in four seasons during a year. Culture-based microbiological analysis was carried out using selective media. Because eukaryotic microflora was found to be negligible, culture-independent analysis was focused to prokaryotes, using 16S rDNA amplification coupled to high throughput sequencing on Illumina MiSeq platform (Illumina, San Diego, California, USA). Aroma-active compounds were extracted by headspace solid-phase microextraction and subsequently analysed by gas chromatography-olfactometry supported by gas chromatography-mass spectrometry. Microflora was found to be dominated by *Lactococcus* spp. with significant levels of *Streptococcus* spp., *Lactobacillus* spp. and *Enterococcus* spp. Dominant aroma-active compounds comprised butanoic acid, diacetyl and 3-methylbutanoic acid, the latter being profound in “nite“ produced from unpasteurized ewes’ milk. Traditionally produced cheeses contained a more diverse prokaryotic microflora and had a stronger aroma profile containing a rich complex of volatile aroma-active compounds, while “nite“ produced from pasteurized cows’ milk by industrial process contained a uniform microflora and had a weaker aroma profile.

Keywords

cheese; lactic acid bacteria; high throughput sequencing; gas chromatography-olfactometry

“Nite“, “vojky“ and also “korbáčiky“ are various forms of traditional Slovakian cheeses, with characteristic organoleptic properties imparted by the fibrous texture and the shape of strings, strands or a whip. These cheeses are very popular in Slovakia, being sold in supermarkets, shops with traditional food products, markets and even in refrigerating automatic selling machines in the streets. These cheese products are usually consumed as snacks.

This type of cheese consists of strings or strands 10–70 cm long and 2–16 mm thick, which are either loose (in case of the thinner “nite“ or thicker “vojky“) or plaited together into the shape of a little whip (in case of “korbáčiky“). It is produced from a partially fermented lump cheese of pH 5.1–5.2, made from unpasteurized cows‘ milk, pasteurized cows’ milk with lactic acid bacterial cultures or unpasteurized ewes’ milk. The lump cheese is cut to pieces, immersed in hot water (63–82 °C) and stirred until it forms an elastic mass. The mass is kneaded, stretched and folded to form a supple, smooth-textured cheese mass, which is then stretched into strands by hand or

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by using two grooved rolling pins rotated against each other. The formed cheese strands are dropped immediately into cold drinking water. After cooling for 2–10 min, the cheese strands are wound onto a reel, cut to the final length and tied with one cheese strand. Then they are immersed in a saturated NaCl solution and hung up to remove excess water. In case of "korbáčiky", the cheese strands are plaited into the shape of a little whip along 2/3 of their length and fastened together by one of the strands, with no change regarding the chemical or microbiological composition. Smoked varieties of the cheese are smoked by means of direct hardwood cold smoke in a smoking chamber. Taste and aroma of these cheeses are mild, slightly acidic and salty. If less salty, they would resemble caciocavallo or scamorza cheeses. Microflora of these cheeses, which is responsible for the production of lactic acid and aroma-active compounds, comprises mainly lactic acid bacteria (LAB) from genera *Lactococcus*, *Streptococcus* and *Lactobacillus.* "Vojky" produced in traditional way in the village of Zázrivá (Slovakia) and "korbáčiky" produced in the region of Orava (Slovakia) have a status of Protected Geographical Indication [1, 2].

The cheeses are produced in industrial and traditional ways. Industrially are produced mainly "nite", which utilize pasteurized milk and starter cultures for production of lump cheeses. Traditional production, products of which are usually accepted as tastier and aromatic, utilizes pasteurized or unpasteurized cows' milk or unpasteurized ewes' milk to produce lump cheeses.

The aim of this study was to characterize microorganisms and key aroma-active compounds in unsmoked "nite" and "vojky" cheeses produced in different ways by three producers from pasteurized cows' milk or unpasteurized ewes' milk. In order to cover the seasonal variability, cheeses were analysed in four seasons during a year.

MATERIALS AND METHODS

Cheese samples

Cheese samples were obtained directly from producers from Slovakia in evacuated polyethylene packages of 100–300 g. Samples were stored at 4 °C and analysed within 3 days with the exception of molecular-biological analysis, for which the samples were frozen at –20 °C and processed within 4 weeks. Samples A were "nite" produced from pasteurized cows' milk in an industrial way. Samples B were "vojky" produced from pasteurized cows' milk in traditional way, with lump cheeses obtained from various producers. Samples C were "nite" produced from unpasteurized ewes' milk in traditional way, with lump cheeses produced in one production facility from one milk source. The samples were collected in four subsequent year seasons (in December 2016, March 2017, June 2017 and August 2017).

Determination of chemical parameters of cheeses

Dry matter was determined according to ISO 5534:2004 [3]. Fat was determined according to STN 57 0107:1965 [4]. Proteins were determined according to ISO 8968-1:2014 [5]. NaCl was determined according to STN 57 0107-12:1980 [6].

Culture-based microbiological analysis

Total aerobic counts were determined according to ISO 4833-1:2013 [7]. Coliforms were determined according to ISO 4832:2006 [8]. Coagulase-positive staphylocci were determined according to ISO 6888-2:1999 [9]. Yeasts and moulds were determined according to ISO 6611:2004 [10]. Presumptive lactobacilli were determined by enumeration of colonies after anaerobic culturing on de Man, Rogosa and Sharpe (MRS) agar (Merck, Darmstadt, Germany) for 72 h at 37 °C. Presumptive lactococci were determined by enumeration of colonies after aerobic culturing on M17 agar (Merck) for 72 h at 30 °C.

Culture-independent microbiological analysis

DNA was isolated from cheese samples by liquid-phase extraction using DNeasy Mericon Food Kit (Qiagen, Hilden, Germany) according to the standard protocol for 200 mg of food sample. Bacterial 16S rDNA fragments were amplified by polymerase chain reaction (PCR) using primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1062R (5'-ACA GCC ATG CAG CAC CT-3') oriented to V1–V6 hypervariable regions [11]. Fragments of the eukaryotic internal transcribed spacer (ITS) were amplified by PCR using primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') oriented to ITS1 and ITS2 [12]. The PCR mixture of 25 μl contained 1.25 U thermostable DNA polymerase (Cheetah Hot Start Taq Polymerase; Biotium, Hayward, California, USA), 1× buffer supplied with the polymerase, 1.5 mmol·l^{-1} $MgCl_2$, 340 μmol·l^{-1} dNTP (Applied Biosystems, Foster City, California, USA) and 300 nmol·l^{-1} of each primer. PCR was carried out in a Veriti thermal cycler (Applied Biosystems) using a programme, for 16S rDNA fragment amplification, consisting of initial denaturation at 94 °C for 2 min, 35 cycles (denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min and poly-

merization at 72 °C for 2 min) and final polymerization at 72 °C for 10 min. For amplification of ITS region, a programme consisting of initial denaturation at 94 °C for 2 min, 35 cycles (denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min and polymerization at 72 °C for 1 min) and final polymerization at 72 °C for 10 min was used. Amplified products were analysed by agarose gel electrophoresis to check the size and amount of the amplified product. Products of PCR were purified by QIAquick PCR Purification Kit (Qiagen), diluted to equimolar ratio and used as template for library preparation using Nextera XT library preparation kit (Illumina, San Diego, California, USA) according to the standard protocol. Samples were analysed using paired-end (2 × 300 bp) sequencing on MiSeq platform (Illumina). Sequencing data were imported into CLC Genomics Workbench Version 7.5 (Qiagen). Each sequence of sample was treated by merging and trimming. Limit of trimming using quality score was set to 0.001 and reads shorter than 150 nucleotides were discarded. Reads were identified based on their homology to reference *16S rRNA* genes in DNA sequence database of National Center for Biotechnology Information (Bethesda, Maryland, USA) using Basic Local Alignment Search Tool (BLAST). BLAST results were processed by MEtaGenome ANalyzer (MEGAN V5; University of Tübingen, Tübingen, Germany) [13].

Analysis of volatile aroma-active compounds

A fraction of volatile compounds was extracted from individual cheese samples of 5.0 g by static incubation in a 40 ml vial in a metallic block thermostat at 50 °C for 30 min, with a solid phase microextraction (SPME) fibre (2 cm) placed in the headspace above the sample. The SPME fibre DVB/Carboxen/PDMS "For odours", film thickness 50/30 μm (Supelco, Bellefonte, Pennsylvania, USA), was used. The fibre was initially conditioned by heating in the injector block of the gas chromatograph at 250 °C for 1 h. SPME sample was then directly thermally desorbed at 250 °C in the injector block of the gas chromatograph.

The volatile compounds extracted by SPME were analysed by gas chromatography-olfactometry (GC/FID-O) in five replicates, using the concept of detection frequency of posterior assessment as described in our previous study [14]. A sniffing procedure panel was formed of 5 judges (2 men, 3 women, aged 29, 47, 50, 57 and 61) who were chosen from 11 assessors trained in sensory evaluation. Results of GC/FID-O analyses were expressed as average values of odour intensity in a scale from 0 to 3 with increments of 0.5, obtained from 5 independent measurements, complying with the requirement of 4 citations within every sensory perception. The gas chromatograph Agilent 7890A (Agilent Technologies, Palo Alto, California, USA) was coupled to a flame ionization detector and to an olfactory detector port ODP3 (Gerstel, Mülheim an der Ruhr, Germany). The capillary column was DB-WAX (30 m × 0.32 mm × 0.25 μm; Agilent Technologies) operated with a temperature programme 50 °C (1 min), 5 °C·min^{-1}, 240 °C (1 min). Hydrogen was used as a carrier gas at a linear velocity of 45 cm·s^{-1} (measured at 143 °C). Pulse splitless injection was used at an injector temperature of 250 °C. The olfactory detector port (ODP) operated at a temperature of 180 °C, interface temperature was 230 °C and the flow of added nitrogen in ODP humidifier was 12 ml·min^{-1}. The sniffing time of each judge did not exceed 30 min.

In order to identify the separated compounds, samples were analysed in parallel by gas chromatography-mass spectrometry (GC-MS) using the gas chromatograph Agilent 6890N (Agilent Technologies) coupled to the mass spectrometric detector 5973 inert (Agilent Technologies) equipped with a column DB-WAXetr (30 m × 0.25 mm × 0.50 μm; Agilent Technologies) operating with a temperature programme 50 °C (1 min), 5 °C·min^{-1}, 240 °C (1 min). The linear velocity of carrier gas helium was 35 cm·s^{-1} (measured at 143 °C). Pulse splitless injection was used at an injector temperature of 250 °C. Ionization voltage (*EI*) was 70 eV.

Individual volatile aroma-active compounds were identified based on comparison of their linear retention indices, mass spectra, analysis of standards, and by comparison of data on occurrence and odour description with literature, as described in our previous studies [14, 15]. Linear retention indices (*LRI*) for individual compounds were calculated, confirmed and compared with *LRI* data obtained by measurement of C_5–C_{13} alkanes as reference standards. For this purpose, our in-house database of *LRI* data was used. Identification of compounds by comparison of mass spectra was done using Registry of Mass Spectral Data (Wiley, New York, New York, USA) and Mass Spectral Library (National Institute of Standards and Technology, Gaithersburg, Maryland, USA).

RESULTS AND DISCUSSION

Chemical parameters of "nite" and "vojky" cheeses from three producers, manufactured in

Tab. 1. Chemical parameters of the studied cheeses.

Parameter	Content [g·kg^{-1}]											
	Autumn			Winter			Spring			Summer		
	A	B	C	A	B	C	A	B	C	A	B	C
Dry matter	505.4	420.9	542.8	506.3	524.9	562.1	498.8	436.9	528.2	480.2	483.1	552.4
Fat	185.0	115.0	267.5	192.5	175.0	270.0	182.5	125.0	260.0	195.0	172.5	297.5
Fat in dry matter	366.0	273.2	492.8	380.2	333.4	480.3	365.9	286.1	492.2	406.1	357.1	538.6
Proteins	257.0	241.6	207.1	262.2	287.6	232.0	259.2	285.9	219.5	249.3	256.2	212.3
NaCl	20.5	26.2	19.8	20.3	23.7	29.5	20.2	27.5	36.7	17.2	22.4	21.6

A – pasteurized cows‘ milk, industrial production; B – pasteurized cows’ milk, traditional production; C – unpasteurized ewes’ milk, traditional production.

four seasons of the year, are summarized in Tab. 1 and demonstrate conformity with official specification [1, 2]. The results demonstrate a comparatively low variability of these products along the year, with the highest fat content in ewes’ milk-based cheeses (samples C), which corresponds to the higher fat content in ewes’ than in cows’ milk [16]. The determined NaCl content in the range of 17.2–29.2 g·kg^{-1} suggests that producers attempt to keep NaCl content lower than the maximum of 45–55 g·kg^{-1} allowed by official specification [1, 2].

Culture-based microbiological parameters of the studied cheeses are summarized in Tab. 2. The results demonstrate the expected highest stability in microbiological quality in case of “nite“ produced from pasteurized cows’ milk in an industrial way (samples A). These cheese samples contained presumptive lactococci as the dominant microorganisms at levels in the order of 10^2–10^3 CFU·g^{-1}, accompanied in summer and autumn by presumptive lactobacilli in the order of 10^2 CFU·g^{-1}. Levels of indicator coliforms were appreciably low along the entire year, with the highest value in the order of 10^2 CFU·g^{-1} in spring. Levels of potentially toxinogenic coagulase-positive staphylococci were low (< 50 CFU·g^{-1}) along the entire year. Moulds were detected at very low levels (< 10–10 CFU·g^{-1}) similar to yeasts, which were more abundant only in spring (in the order of 10^2 CFU·g^{-1}).

Clearly higher levels of LAB were determined in “vojky“ produced from pasteurized cows’ milk in traditional way (samples B), with dominant presumptive lactococci in the order of 10^3–10^8 CFU·g^{-1} always being accompanied by presumptive lactobacilli (in the order of 10^3–10^5 CFU·g^{-1}). Also in these samples, coliforms were low along the entire year, with the highest value in the order of 10^2 CFU·g^{-1} in spring, and levels of potentially toxinogenic coagulase-positive staphylococci were low (< 50 CFU·g^{-1}) along the entire year. Moulds were detected at low levels (< 10–10 CFU·g^{-1}) and yeasts were also low (< 10–10 CFU·g^{-1}), with greater contents detected in autumn and spring, in the order of 10^2 CFU·g^{-1}.

Much more microorganisms were detected in “nite“ produced from unpasteurized ewes’ milk (samples C), which contained high levels of presumptive lactococci (in the order of 10^6–10^8 CFU·g^{-1}) and presumptive lactobacilli (in the order of 10^6–10^7 CFU·g^{-1}) accompanied with considerable levels of coliforms (in the order of 10^3–10^6 CFU·g^{-1}). In these samples, coagulase-positive staphylococci were detected at levels in the order of 10^2–10^3 CFU·g^{-1}. Levels of moulds ranged in the order of 10^1–10^3 CFU·g^{-1} and levels of yeasts ranged in the order of 10^2–10^5 CFU·g^{-1}.

The results of culture-based microbiological analysis demonstrated the overall success of the technological approach based on the use of pasteurized milk and strictly controlled process conditions to obtain a safe and microbiologically reproducible product. However, levels of LAB in such product can be taken as low, compared to those in “vojky“ produced from pasteurized cows’ milk in traditional way (samples B). “Nite“ produced from unpasteurized ewes’ milk (samples C) were clearly superior in the contents of LAB but contained also certain levels of hygienically or technologically unfavourable microorganisms, which were not suppressed by treatment with hot water. The determined contents of microorganisms were comparable to the previously published data on ewes’ lump cheese [17], May bryndza cheese [18] or similar cheeses [19] and complied with legislative requirements [20].

Culture-based data were supplemented by culture-independent analysis using high throughput sequencing. Eukaryotic microflora was analysed in autumn samples, where unidentified Ascomycota were found dominant in sample B, *Dipodascus* spp. was found dominant in sample C and ITS amplification failed in sample A, probably because of

Tab. 2. Culture-based microbiological parameters of the studied cheeses.

Microorganisms	Content [CFU·g^{-1}]											
	Autumn			Winter			Spring			Summer		
	A	B	C	A	B	C	A	B	C	A	B	C
Total aerobic counts	8.2×10^2	3.1×10^4	$> 3.0 \times 10^7$	2.3×10^3	2.8×10^6	$> 3.0 \times 10^7$	2.3×10^4	2.3×10^5	$> 3.0 \times 10^8$	2.1×10^4	2.9×10^8	$> 3.0 \times 10^8$
Coliforms	< 10	30	$> 1.5 \times 10^6$	70	20	6.1×10^3	5.1×10^2	1.0×10^2	9.7×10^4	10	< 10	1.2×10^4
CP staphylococci	< 50	< 50	2.0×10^3	< 50	< 50	1.0×10^2	< 50	< 50	7.5×10^2	< 50	< 50	5.0×10^2
Yeasts	< 10	6.1×10^2	2.1×10^4	< 10	< 10	1.0×10^5	3.2×10^2	2.4×10^2	5.7×10^2	40	10	1.7×10^3
Moulds	10	10	3.9×10^3	< 10	< 10	3.8×10^2	< 10	< 10	10	< 10	< 10	60
Presumptive lactobacilli	3.9×10^2	2.8×10^3	2.4×10^6	< 10	7.1×10^4	2.0×10^6	60	2.0×10^3	9.0×10^6	1.1×10^2	3.4×10^5	2.9×10^7
Presumptive lactococci	2.0×10^2	4.9×10^3	$> 3.0 \times 10^6$	1.3×10^2	3.0×10^5	$> 3.0 \times 10^7$	1.1×10^3	4.6×10^4	$> 3.0 \times 10^8$	1.2×10^3	1.9×10^8	$> 3.0 \times 10^8$

A – pasteurized cows‘ milk, industrial production; B – pasteurized cows’ milk, traditional production; C – unpasteurized ewes’ milk, traditional production; CP – coagulase-positive.

Tab. 3. Percentual share of prokaryotic taxons in cheeses as determined by culture-independent approach.

Taxon	Share [%]											
	Autumn			Winter			Spring			Summer		
	A	B	C	A	B	C	A	B	C	A	B	C
Lactococcus spp.	98.4	88.4	48.2	98.7	76.3	2.0	97.1	84.6	75.3	96.0	80.3	92.4
Streptococcus spp.	0.5	5.7	5.2	0.1	1.9	74.1	0.7	2.2	8.4	1.5	7.2	3.9
Lactobacillus spp.	0.0	3.6	0.1	0.0	20.4	0.1	0.0	0.3	0.2	0.0	9.4	0.0
Leuconostoc spp.	0.0	0.7	0.0	0.0	0.0	0.0	0.0	9.7	0.0	0.0	0.2	0.0
Enterococcus spp.	1.1	1.5	0.9	1.0	1.0	0.5	1.9	2.5	2.0	2.3	2.4	2.1
Enterobacteriaceae	0.0	0.0	21.3	0.0	0.0	5.2	0.0	0.0	11.7	0.0	0.0	0.6
Pseudomonas spp.	0.0	0.0	0.2	0.0	0.1	0.1	0.2	0.0	0.2	0.0	0.1	0.1
Staphylococcus spp.	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.1	0.0	0.0	0.2
Other or unassigned	0.0	0.1	24.1*	0.2	0.3	16.7	0.1	0.7	2.1	0.2	0.4	0.7
Sequences total	320077	164890	254692	79189	101731	103143	11166	17333	16043	159406	128063	127104

A – pasteurized cows‘ milk, industrial production; B – pasteurized cows’ milk, traditional production; C – unpasteurized ewes’ milk, traditional production.
* – *Acetobacter* spp. and *Gluconacetobacter* spp. were dominant.

a very low content of eukaryotic microorganisms (data not shown). Because these expensive analyses were found to be not very effective in producing novel knowledge, culture-independent analysis of eukaryotic microflora was not carried out in further samples. In fact, eukaryotic microflora is not developed in short-ripened cheeses and does not seem to have a significant impact on cheese aroma [19].

Data on culture-independent analysis of prokaryotic microflora are summarized in Tab. 3. With a few exceptions ("nite" from unpasteurized ewes' milk, samples C in autumn and winter), *Lactococcus* spp. were dominant in all cheese samples, accounting for > 75 % amplicons. This agreed in particular with *Lactococcus* spp. being the main component of starter cultures used for production of lump cheese from pasteurized milk, but being widely present in raw milk and rapidly propagated during first days of lump cheese ripening [17, 19]. The second widest detected LAB was *Streptococcus* spp., which was also present in all cheese samples in all year seasons. It is another component of starter cultures, which explains its presence in cheeses made from pasteurized milk (samples A, B). Its presence and even dominance in "nite" made from unpasteurized ewes' milk (samples C) can be explained within the great microbiological variability of this cheese, reflecting the occasional use of internal whey cultures. Another widely detected LAB was *Lactobacillus* spp., which was absent from cheeses produced from pasteurized milk in industrial way (samples A), but present in all cheeses produced from pasteurized milk in traditional way (samples B) and in cheeses from unpasteurized ewes' milk (samples C) produced in three out of four seasons. Absence of this LAB in industrially produced cheeses suggests the use of different starter cultures than in traditional production. Different starter cultures may be responsible also for the exclusive presence of *Leuconostoc* spp. in cheeses from pasteurized milk produced in traditional way (samples B) in three out of four year seasons. Presence of *Enterococcus* spp. in all cheese samples, including those made from pasteurized milk, reflects the thermal resistance of this microorganism and its intensive growth in cheeses [21].

Culture-independent microbiological analysis provided interesting information also on prokaryotic contaminants of the cheeses. Enterobacteriaceae, which are taken as indicators of hygienic problems, were found exclusively in cheeses produced from unpasteurized ewes' milk (samples C) in all year seasons. These results demonstrated good manufacturing practice applied at the production of cheeses A and B, which avoided post-pasteurization contamination by Enterobacteriaceae. Cheeses produced from pasteurized cows' milk were also devoid of *Staphylococcus* spp., which is an important toxinogenic contaminant of cheeses. Although it was detected in all cheeses from unpasteurized ewes' milk, confrontation with data obtained by culture-based methods showed that *Staphylococcus* spp. did not reach hygienically unacceptable levels. These results confirmed the success of suppression of growth of *Staphylococcus* spp. by the activity of LAB in the production process of cheeses [22]. Majority of cheeses were contaminated by *Pseudomonas* spp., which was uncommon in case of "nite" from pasteurized cows' milk (sample A) industrially produced in spring. While this microorganism may originate in environmental contamination during traditional production, its presence in industrially produced cheese rather suggests psychrophilic selection, e.g. using longer refrigerated milk or longer refrigerated lump cheese [23].

Overall good agreement was observed with data obtained by culture-independent and culture-based microbiological analyses. However, some controversy was observed with *Lactobacillus* spp., which was found absent, by culture-independent analysis, in cheeses produced from pasteurized milk in an industrial way (samples A), while presumptive lactobacilli were detected in the order of 10^2–10^5 CFU·g^{-1} by culture-based analysis. Since *Lactobacillus* spp. was detected at reasonable levels by the culture-independent approach in all "vojky" cheeses produced from pasteurized cows' milk in traditional way (samples B) and in three out of four "nite" cheeses produced from unpasteurized ewes' milk (samples C), the culture-independent method appeared to be sensitive enough and we interpret this observation as false positive detection of non-*Lactobacillus* colonies on MRS agar, due to insufficient selectivity of this medium.

Results of the analysis of aroma-active compounds are summarized in Tab. 4, Tab. 5 and Tab. 6. In cheeses produced from pasteurized cows' milk in industrial way (samples A), 42 odour zones were recognized by GC/FID-O throughout entire year, including two odouric co-elutions (Tab. 4). Diacetyl, acetic acid, dimethyl sulfone, nonanoic acid and δ-decalactone were key odourants throughout entire year in this series, however, their intensities were comparatively low, 1.5–2 at the utmost. Further odourants were recorded sporadically in various year seasons, often at low odour itensities, dominantly 0.5. These cheese samples lacked the presence of several typical cheese odourants such as ethylacetate,

Tab. 4. Key volatile aroma-active compounds in cheeses produced from pasteurized milk in industrial way (samples A).

No.	Aroma compound	Aroma description	Aroma intensity				Basis for identification
			Autumn	Winter	Spring	Summer	
1	Acetaldehyde + dimethylsulfide[t]	Unpleasant, rotten sulfurous odour, slightly cheesy	0.5	1[a]	0.5	0.5	MS, OD, LIT
2	2-Butanone	Ethereal, acetone-like	0.5[a]		0.5	2[a]	MS, OD, LIT
3	Ethanol	Alcoholic, ethereal			1		MS, OD, LIT
4	2,3-Butanedione(diacetyl)	Cottage cheese, acidophilus milk, sour cream, whey-like	1.5	2	1.5		MS, LRI, ST, OD, LIT
5	α-Pinene	Earthy, herbal	0.5	–	–	–	MS, LRI, ST, OD, LIT
6	Toluene	Solvent-like with sweetish, slightly fruity odour	0.5	–	–	0.5	MS, OD, LIT
7	D-Limonene	Plant, slightly bitterish, fresh citrus-like	0.5	0.5	–	0.5	MS, LRI, ST, OD, LIT
8	3-Methyl-2-butenal	Fruity, pleasant	–	0.5	–	–	MS, OD, LIT
9	β-Phellandrene	Minty, terpenic	0.5	–	–	–	MS, OD, LIT
10	Unknown°	Fruity, pleasant	0.5	–	–	–	–
11	Styrene(vinylbenzene)	Pleasant, sweetish, slightly plastic-like	0.5	–	–	0.5	LRI, ST, OD, LIT
12	*p*-Cymene	Terpenic, fuel-like	0.5	0.5	–	–	MS, LRI, ST, OD, LIT
13	Acetoin (3-hydroxy-2-butanone)	Pleasant, sweetish, milky	0.5	0.5	0.5	–	MS, LRI, ST, OD, LIT
14	Hexanol	Plant-like, bitterish	–	0.5	–	–	LRI, ST, OD, LIT
15	Unknown°	Sweetish, slightly solvent-like	–	0.5	–	–	–
16	Unknown°	Plastic-like, rubber-like odour	0.5	–	–	–	–
17	Acetic acid	Vinegar-like, soury	2	1.5	1.5	1.5	MS, OD, LIT
18	Methional (3-methylthio-propanal)	Potato-like, earthy	0.5	–	–	–	LRI, ST, OD, LIT
19	Furfural	Fragrant, sweetish, baked	–	–	–	0.5	MS, LRI, ST, OD, LIT
20	Unknown°	Sweetish, fruity	0.5	0.5	–	–	–
21	Butanoic acid	Unpleasant, sweaty, cheesy	–	1.5	1.5	–	MS, OD, LIT
22	Methylformamide	Ethereal, slightly cheesy-like	0.5	–	–	–	MS, OD, LIT
23	γ-Butyrolactone[t]	Pleasant, sweetish, creamy	–	–	1[a]	–	MS, OD, LIT
24	2-Furanmethanol	Irritating, pungent, burnt caramel-like odour, bitterish plants-like	–	1		–	MS, LRI, ST, OD, LIT
25	3-Methylbutanoic acid (isovaleric acid)	Unpleasant, rotten cheese-like	–	–	–	0.5	MS, LRI, ST, OD, LIT
26	D-Carvone	Fresh, minty	–	–	1	–	LRI, ST, OD, LIT
27	Methoxyphenyl oxime[t]	Cooked potato-like, earthy, plant-like	0.5	0.5	0.5	0.5	MS, OD, LIT
28	Unknown°	Mastic-like, putty, paint-like	1	–	–	–	–
29	Hexanoic acid + unknown°	Irritating in nose, sour, slightly cheesy, fatty, unpleasant	–	–	1[a]	1	MS, OD, LIT
30	Dimethylsulfone	Burnt, smoky	1	1		1.5	MS, OD, LIT
31	2-Phenylethanol	Fragrant sweetish, flowery, rose odour	1	0.5	–	0.5	MS, LRI, ST, OD, LIT
32	Unknown°	Unpleasant, musty	0.5	–	–	–	–
33	Unknown°	Earthy, cooked potatoes-like	–	–	0.5	–	–
34	Phenol[t]	Sweet, burnt caramel-like, slight tarry-like	1	1.5	1.5	–	MS, OD, LIT
35	Octanoic acid	Unpleasant fatty-waxy, plastic-like, exhaust gases-like	0.5	1	1.5	–	MS, OD, LIT

Tab. 4. *continued*

No.	Aroma compound	Aroma description	Aroma intensity				Basis for identification
			Autumn	Winter	Spring	Summer	
36	Unknown^{o}	Sweetish, slight caramel-like	0.5	–	–	–	–
37	Nonanoic acid	Unpleasant, waxy, fatty, overripe cheese-like, plastic, bitter plant-like	1.5	1.5	0.5	1	MS, OD, LIT
38	δ-Decalactone	Fragrant, sweetish, coconut-like, fatty, milky	2	2	2	1.5	LRI, ST, OD, LIT
39	Methyl-(*E*)-dihydrojasmonate (hedion)	Fragrant, pleasant, herbaceous	–	–	–	0.5	LRI, ST, OD, LIT
40	Dodecanoic acid (lauric acid)	Pleasant, fragrant, herbal, bay leaf-like	1	0.5	0.5	–	MS, OD, LIT
41	Tridecanoic acid	Woody, herbaceous, soapy	1	0.5	–	–	MS, OD, LIT

Compounds were identified on the basis of the following criteria: MS – mass spectrum, LRI – linear retention index, ST – comparison with the reference compound, OD – odour quality, LIT – literature reference.
t – tentative identification (only on the basis of mass spectra), o – compound detected only by GC/FID-O, a – co-elution.

Tab. 5. Key volatile aroma-active compounds in cheeses produced from pasteurized milk in traditional way (samples B).

No.	Aroma compound	Aroma description	Aroma intensity				Basis for identification
			Autumn	Winter	Spring	Summer	
1	Dimethylsulfide^{t}	Unpleasant, putrid, slightly sour-cheesy	1	–	–	1	OD, LIT
2	Ethylacetate + 2-butanone	Slight cheese-fruity, acetone-like, ethereal	–	1	–	1	MS, LRI, ST, OD, LIT
3	Benzene	Unpleasant, sweetish, pungent, gasoline-like, petroleum-like	0.5	–	0.5	1	MS, OD, LIT
4	Ethanol	Solvent-like, alcoholic, ethereal	1	–	–	–	MS, OD, LIT
5	2-Pentanone + 2,3-butanedione(diacetyl)	Cottage cheese, acidophilus milk, sour cream, whey-like	1.5	2	1.5	2	MS, LRI, ST, OD, LIT
6	2-Butanol	Sweetish, organic solvent-like, slightly irritating	–	–	1	–	LRI, ST, OD, LIT
7	Ethylbutanoate	Sweetish, fruity	0.5	0.5	0.5	0.5	MS, LRI, ST,OD, LIT
8	Alpha-thujene^{t}	Woody, herbal, slightly bitterish	–	0.5	–	–	MS, OD, LIT
9	Unknown^{o}	Pleasant, sweet, fruity	1	–	–	–	OD, LIT
10	Toluene	Solvent-like with sweetish odour	–	1	–	–	MS, OD, LIT
11	Unknown^{o}	Plant-like, fresh, grassy	–	1	0.5	–	–
12	2-Methyl-1-propanol	Wine-like, fusel alcohol, slightly fruity, plant, solvent-like	0.5	0.5	0.5	–	MS, LRI, ST, OD, LIT
13	Unknown^{o}	Sweetish, caramel-fruity	1	–	0.5	–	–
14	Unknown^{o}	Sweetish, fruity	–	1	0.5	–	–
15	2-Heptanone	Pleasant,sweetish, caramel-like, herbal, spicy	0.5	1	–	–	MS, LRI, ST, OD, LIT
16	D-limonene	Fresh citrus-like	–	–	–	0.5	MS, LRI, ST, OD, LIT
17	Unknown^{o}	Sweetish, fruity	–	–	–	0.5	–
18	3-Methylbutanol	Unpleasant, fermented sour stink, rotten yeast	–	0.5	–	–	MS, LRI, ST, OD, LIT
19	Ethylhexanoate	Fruity, wine-like, green apple, slightly sweetish, brandy	0.5	–	0.5	–	MS, LRI, ST, OD, LIT
20	1-Pentanol	Pleasant, sweetish	–	1	–	–	MS, LRI, ST, OD, LIT
21	Styrene (vinylbenzene)	Pleasant, sweetish, slightly tabacco-like	–	–	1.5	0.5	MS, LRI, ST, OD, LIT

Tab. 5. *continued*

No.	Aroma compound	Aroma description	Aroma intensity				Basis for identification
			Autumn	Winter	Spring	Summer	
22	Acetoin (3-hydroxy 2-butanone) + octanal	Pleasant, sweetish, milky, creamy, fatty, tallowy	1	1	–	0.5	MS, LRI, ST, OD, LIT
23	3-Methyl-2-butenol (prenol)	Fruity, pleasant	–	–	–	0.5	MS, LRI, ST, OD, LIT
24	Unknown°	Dairy, pleasant	–	–	1	–	–
25	Unknown°	Burnt, smoky, tobacco-like, irritating the nose	1	0.5	–	–	–
26	6-Methyl-5-hepten-2-one	Herbaceous, green, oily	–	–	–	0.5	MS, LRI, ST, OD
27	3-Pentanol	Herbal, oily, soap-like	–	1.5	–	–	MS, OD, LIT
28	Unknown°	Fresh cheesy, cottage cheese	1	–	–	–	–
29	2-Nonanone	Plant-like, fresh, floral	–	0.5	–	–	MS, LRI, ST, OD, LIT
30	Nonanal	Strange mixed odour, plant-like, sweetish, fruity, slightly waxy	–	1	–	–	MS, LRI, ST, OD, LIT
31	Acetic acid	Vinegar-like, soury	1	2	2	1.5	MS, OD, LIT
32	Methional (3-methylthio-propanal)	Potato-like, earthy	–	1	–	–	LRI, OD, LIT
33	Decanal	Citrus-like, sweetish, slightly waxy	–	–	–	1	MS, LRI, ST, OD, LIT
34	Isovanillin	Phenolic, medicinal	1	–	–	–	MS, OD, LIT
35	2,3-Butanediol (unknown diasteroisomer)	Sweetish, caramel-like	1	–	–	–	MS, OD, LIT
36	Benzaldehyde	Herbaceous, bitterish	–	0.5	–	–	MS, LRI, ST, OD, LIT
37	2,3-Butanediol (unknown diasteroisomer)	Fragrant, sweetish	1.5	–	–	–	MS, OD, LIT
38	Butanoic acid	Unpleasant, fatty, cheesy	0.5	2	–	2	MS, OD, LIT
39	1-Nonanol	Floral, fresh, clean, orange	–	–	–	1	MS, LRI, ST, OD, LIT
40	Acetophenone	Pleasant, sweetish, floral, slightly almond-like, vanilla-nuance	–	–	–	1	MS, OD, LIT
41	D-carvone	Fresh, cool, peppermint-like	–	–	0.5	1	LRI, ST, OD, LIT
42	Unknown°	Dried plum, sweetish	–	1	–	–	–
43	Methoxyphenyl oxime	Cooked potato, earthy, roasty	0.5	0.5	1	1	MS, OD, LIT
44	Unknown°	Sweetish, baked	–	–	1	–	–
45	Hexanoic acid + unknown°	Irritating the nose, sour, slightly cheesy, fatty, unpleasant	1	1	–	–	MS, OD, LIT
46	Dimethylsulfone	Burnt, smoky	1.5	2	2	2	MS, OD, LIT
47	2-Phenylethanol	Fragrant, flowery, rose odour	1	0.5	–	–	MS, LRI, ST, OD, LIT
48	Unknown°	Smoky, spicy, herbal, woody	1	1	1	1.5	–
49	Tuberolide (dihydro-4-methyl-5-pentyl-2(3h)-furanone)[t]	Mixed odour dairy-fatty, lactonic, caramel-like	–	1	1.5[a]	0.5[a]	MS, OD
50	Phenol	Sweet, burnt caramel-like, slightly tarry-like	1.5	1.5			MS, OD, LIT
51	Octanoic acid	Unpleasant, rancid oily, fatty, plastic-like, exhaust gases-like	0.5	1	1.5	1	MS, OD, LIT
52	Thymol (2-isopropyl-5-methylphenol)	Thyme, herbal, spicy, woody	0.5	–	–	1	MS, OD, LIT
53	Unknown°	Caramel-like	–	–	–	1	–
54	Unknown°	Sweet, caramel-like	0.5	0.5	–	1	–

Tab. 5. *continued*

No.	Aroma compound	Aroma description	Aroma intensity				Basis for identification
			Autumn	Winter	Spring	Summer	
55	Nonanoic acid	Unpleasant, waxy, fatty, plastic, bitterish, slightly citrus peel-like	1.5	1.5	1	0.5	MS, OD, LIT
56	δ-Decalactone	Fragrant, sweetish, coconut-like, fatty, milky	2.5	2	2	2	MS, LRI, ST, OD, LIT
57	Decanoic acid (capric acid)	Unpleasant milky-fatty, slightly sour, citrus-like	1	0.5	–	–	MS, OD, LIT
58	Methyl-(*E*)-dihydrojasmonate (hedion)	Pleasant herbaceous, vegetable, soapy	0.5	1	0.5	–	MS, LRI, ST, OD, LIT
59	Unknown^{o}	Fresh cheese-like	0.5	0.5	–	1.5	–
60	Benzoic acid	Pleasant, fragrant herb	0.5	0.5	1	1	MS, OD, LIT
61	Dodecanoic acid (lauric acid)	Pleasant, fragrant, herbal, bay leaf-like	0.5	1	1	0.5	MS, OD, LIT
62	Tridecanoic acid	Woody, herbaceous, soapy	–	0.5	–	–	MS, OD, LIT

Compounds were identified on the basis of the following criteria: MS – mass spectrum, LRI – linear retention index, ST – comparison with the reference compound, OD – odour quality, LIT – literature reference.
t – tentative identification (only on the basis of mass spectra), o – compound detected only by GC/FID-O, a – co-elution.

Tab. 6. Key volatile aroma-active compounds in cheeses produced from unpasteurized ewes' milk in traditional way (samples C).

No.	Aroma compound	Aroma description	Aroma intensity				Basis for identification
			Autumn	Winter	Spring	Summer	
1	1,3-Pentadiene (unknown isomer) + acetaldehyde	Unpleasant, acrid, kerosene-like, ethereal, musty	1	1	–	–	MS, OD, LIT
2	2-Methylbutanal+3-methylbutanal (isovaleraldehyde)	Unpleasant, fermented, rotten cheese-like	1	1.5	1.5	2.5	LRI, MS, OD, LIT
3	2-Pentanone	Sweet milk-like	1	–	–	–	LRI, MS, OD, LIT
4	2,3-Butanedione (diacetyl)	Cottage cheese, acidophilous milk, sour cream, whey-like	2	2	2	3	LRI, MS, OD, LIT
5	Toluene	Organic solvent with sweetish odour	–	1	–	0.5	MS, OD, LIT
6	2-Methylpropanol (isobutylalcohol)	Weak fruity, wine-like	0.5	0.5	–	0.5	LRI, MS, OD, LIT
7	*p*-Xylene	Sweetish-aromatic solvent odour	0.5	0.5	–	–	LRI, MS, OD, LIT
8	2-Heptanone + D-limonene	Pleasant, sweetish, fruity	–	0.5	–	0.5	LRI, MS, OD, LIT
9	3-Methylbutanol	Unpleasant, fermented sour stink, rotten yeast	1	2	2	2	LRI, MS, OD, LIT
10	Styrene (vinylbenzene)	Balsamic, sweet	–	1	–	–	LRI, MS, OD, LIT
11	Acetoin (3-hydroxy 2-butanone) + unknown^{o}	Buttery, creamy, pleasant fresh, slightly citrus-like	0.5	0.5	1.5	2^{a}	LRI, MS, OD, LIT
12	Octanal	Bitterish, fatty, unpleasant	0.5	–	0.5		LRI, MS, OD, LIT
13	Unknown^{o}	Fruity, floral	–	0.5	–	–	–
14	Unknown^{o}	Fruity, sweetish	0.5	0.5	0.5	1	–
15	Hexanol	Bitterish, plant-like, green	1	1	1	–	LRI, MS, OD, LIT
16	2-Hydroxy-3-pentanone^{t}	Truffle, nutty	1	0.5	–	–	MS, OD, LIT
17	2-Nonanone	Sweetish, fruity, pleasant	0.5	–	–	–	LRI, OD, LIT
18	Nonanal	Waxy, bitterish plant, citrus peel	1	1.5	1	0.5	LRI, OD, LIT

Tab. 6. *continued*

No.	Aroma compound	Aroma description	Aroma intensity				Basis for identification
			Autumn	Winter	Spring	Summer	
19	Acetic acid	Vinegar, pungent sour odour	1.5	1.5[a]	1	1	MS, OD, LIT
20	Methional (3-methylthio propanal)	Cooked potato-like	–		1	–	LRI, OD, LIT
21	Furfural (2-furaldehyde)	Baked bread, fragrant, slightly sweet	–	0.5	–	–	LRI, MS, OD, LIT
22	2-Ethylhexanol	Sweetish, slight rose	1	–	–	–	LRI, MS, OD, LIT
23	2-Acetylfuran	Sweet, slightly burned, cocoa-like, slightly coffee-like	–	–	–	1	LRI, MS, OD, LIT
24	Benzaldehyde	Fragrant, bitter almond, sour cherry-like, pleasant	1	0.5	1	–	LRI, MS, OD, LIT
25	Unknown°	Sweetish, fruity, pleasant	–	0.5	–	–	–
26	2,3-Butanediol (unknown diasteroisomer)	Slightly sweetish	–	–	–	0.5	MS, OD, LIT
27	Butanoic acid	Unpleasant, overripened cheese-like, putrid odour	1.5	2	2	2	MS, OD, LIT
28	Benzeneacetaldehyde (phenylacetaldehyde)	Sweet heavy fragrance, honey-like, rose-like	–	1.5	–	2.5[a]	LRI, MS, OD, LIT
29	3-Methylbutanoic acid (isovaleric acid)	Unpleasant rancid odour, fecal, putrid	3	2	2		LRI, MS, OD, LIT
30	D-carvone	Herbaceous, fresh, spicy, caraway-like, peppermint-like	–	–	1.5	1.5	LRI, MS, ST, OD, LIT
31	Methoxyphenyl oxime[t]	Earthy, cooked potato-like, roasty	0.5	–	1	1	MS, OD, LIT
32	Hexanoic acid	Unpleasant, acrid stink	2	1	1	2.5[a]	MS, OD, LIT
33	2-Methoxyphenol (guaiacol)	Smoky, burnt	1.5	2	2		MS, OD, LIT
34	2-Phenylethanol	Fragrant, flowery, rose odour	1	2	1.5	2.5	LRI, MS, OD, LIT
35	Unknown°	Unpleasant, musty odour	1	–	1	0.5	–
36	Unknown°	Unpleasant, rotten cabbage-like	–	–	–	1	–
37	Phenol + unknown°	Sweet, burnt caramel-like, slightly tarry-like	–	–	1	0.5	MS, OD, LIT
38	Octanoic acid	Unpleasant, rancid oily, fatty, plastic-like, exhaust gases-like	1	1	1	1.5[a]	MS, OD, LIT
39	*p*-cresol	Medicinal, cresylic, phenolic, tarry-smoky, green plant stalks-like	0.5	1	1		LRI, MS, OD, LIT
40	Nonanoic acid	Waxy, fatty, plastic-like	1	1.5	1.5[a]	1.5	MS, OD, LIT
41	δ-decalactone	Fragrant, sweetish, fat, milky, coconut-like	3	1.5		2	LRI, MS, OD, LIT
42	Decanoic acid (capric acid)	Soap-like, fatty, citrus-like	1	1	1	–	MS, OD, LIT
43	Methyl-(*E*)-dihydrojasmonate (hedion)	Fresh floral, jasmine note with citrus freshness	–	0.5	1	1	LRI, MS, OD, LIT
44	Benzoic acid	Pleasant, faint balsamic odour	1	–	1	–	MS, OD, LIT
45	Dodecanoic acid (lauric acid)	Pleasant, fragrant soap-like, fatty	1	1	1	1	MS, OD, LIT
46	Tridecanoic acid	Pleasant, herbaceous, faint floral, fresh soap-like	–	1	–	–	MS, OD, LIT
47	Unknown°	Pleasant, fragrant, fresh, herbaceous	0.5	–	–	–	–
48	Unknown°	Pleasant, sweetish, herbal essential oil-like	–	1	–	–	–
49	Hexadecanoic acid (palmitic acid)	Faint waxy, fatty odour with candle waxy nuance	1	–	–	–	MS, OD, LIT

Compounds were identified on the basis of the following criteria: MS – mass spectrum, LRI – linear retention index, ST – comparison with the reference compound, OD – odour quality, LIT – literature reference.
t – tentative identification (only on the basis of mass spectra), o – compound detected only by GC/FID–O, a – co–elution.

benzene, 2-pentanone, ethylbutanoate, 3-methylpropanol, 2-heptanone, tuberolide (dihydro-4-methyl-5-pentyl-2(H)-furanone) or decanoic acid, which were detected in other cheeses in this study. Cheeses produced from pasteurized cows' milk in industrial way were overall characterized by weaker aroma profiles, and also by weaker taste, as determined by degustation (data not shown).

In cheeses produced from pasteurized cows' milk in traditional way (samples B), a greater number of 65 odour zones were recognized by GC/FID-O throughout entire year, including four odouric co-elutions (Tab. 5). Key odourants were 2-pentanone + diacetyl (2,3-butanedione; co-eluting) at intensities 1.5–2 throughout entire year, acetic acid at intensities 1–2 throughout entire year, butanoic acid at intensities 1.5–2 (undetected in spring), octanoic acid at intensities 0.5–1.5 throughout entire year, nonanoic acid at intensities 0.5–1.5 throughout entire year, dodecanoic acid at intensities 0.5–1 throughout entire year, δ-decalactone at intensities 2–2.5 throughout entire year, benzoic acid at intensities 0.5–1 throughout entire year, dimethylsulfone at intensities 1.5–2 throughout entire year, benzoic ethylbutanoate at intensity 0.5 throughout entire year and methoxyphenyloxime at intensities 0.5–1 throughout entire year. Unknown compound No. 51 was detected at intensities 1–1.5 throughout entire year. Dimethylsulfide and dimethylsulfone were detected in both industrially and traditionally produced cows' milk cheeses, while thymol was detected only in traditionally produced ones in summer and autumn. Comparing cheeses of this series produced along the year, it can be noticed that several specific compounds with sweetish, citrus-like notes were present in summer season, namely, D-limonene, unknown compound No. 17 or 3-methyl-2-butenol (prenol), with herbal, green odours, namely, 6-methyl-5-hepten-2-one, and with sweet-caramel notes, namely, unknown compound No. 56. Regarding other odourants, these were, as a rule, olfactorically detected at highest intensities in summer samples (dimethylsulfide, ethylacetate + 2-butanone, benzene, D-carvone, unknown compound No. 51, thymol, unknown compound No. 57, unknown compound No. 62 and benzoic acid).

In cheeses produced from unpasteurized ewes' milk (samples C), a number of 49 odour zones were recognized by GC/FID-O, including four odouric co-elutions (Tab. 6). Key odourants that were present along the entire year were 2-methylbutanal + 3-methylbutanal (co-elution) at intensities 1–2.5, diacetyl at intensities 2–3, 3-methylbutanol at intensities 1–2, acetoin (3-hydroxy-2-butanone) at intensities 0.5–2, nonanal at intensities 0.5–1.5, acetic acid at intensities 1–1.5, butanoic acid at intensities 1.5–2, 3-methylbutanoic acid at intensities 1–3, hexanoic acid at intensities 1–2.5, 2-phenylethanol at intensities 1–2.5, octanoic acid at intensities 1–1.5, *p*-cresol at intensities 0.5–1.5, nonanoic acid at intensities 1–1.5, δ-decalactone at intensities 1.5–3, decanoic acid at intensity 1 (not detected in summer) and dodecanoic acid at intensity 1. Regarding variability along the year, summer samples were characterized by higher intensities of odourants of unpleasant and "overferemented" notes, respectively, (2-methylbutanal + 3-methylbutanal, 3-methylbutanol, octanal, butanoic, 3-methylbutanoic acid, hexanoic acid + guaiacol (2-methoxyphenol), unknown compound No. 36, octanoic acid, *p*-cresol and nonanoic acid). Several odourants specific for this kind of cheese were detected, namely, 2-methylbutanal + 3-methylbutanal, *p*-cresol, guaiacol (all detected along entire year), 1,3-pentadiene + acetaldehyde and *p*-xylene (detected in autumn and winter). In other year seasons, further odourants specific for this kind of cheese were sporadically detected, namely, hexanol, 2-hydroxy-3-pentanone, furfural (2-furaldehyde), 2-ethylhexanol, 2-acetylfuran and phenylacetaldehyde. 3-Methylbutanoic acid seemed to play a dominant organoleptic role in „nite" cheese produced from unpasteurized ewes' milk in contrast to counterpart cheeses made from cows' milk. An important organoleptic role in the former cheese was identified also for odourants such as hexanoic acid, diacetyl, guaiacol, butanoic acid, δ-decalactone and 2-phenylethanol. The mentioned odourants largely contributed, with their high odour intensities, to the overall aroma of cheeses produced from unpasteurized ewes' milk, being its characteristic components.

A level of correlation was observed between the composition of microflora and the profile of aroma-active compounds of the studied cheeses. In particular, cheeses produced from pasteurized cows' milk in industrial way (samples A) had the weakest and least complex aroma profile, which corresponded to the great dominance of lactococci as typical primary starter bacteria with a limited production of aroma-active compounds. Regarding the two types of cheese produced in traditional way, the effect of pasteurization on the profile of aroma-active compounds seemed less pronounced than the effect of ewes' milk versus cows' milk. This observation may be connected with a different chemical composition of ewes' milk versus cows' milk, or by different microflora bound to individual milk types, despite of pasteurization in

case of cows' milk. These results are in concordance with current knowledge in the field [24–26].

CONCLUSIONS

Three types of "nite" and "vojky" cheese produced in 2016–2017 were characterized with the main focus on diversity of bacteria and key aroma-active compounds. The detailed chemical and microbiological data revealed clear differences between cheeses produced from pasteurized cows' milk in industrial way, cheeses produced from pasteurized cows' milk in traditional way and cheeses produced from unpasteurized ewes' milk in traditional way, with minor variations along the year. The results showed that industrially produced cheeses contained a better defined microflora with a minimum of microorganisms other than lactic acid bacteria, but their profile of aroma-active compounds was inferior. On the other hand, traditionally produced cheeses, in particular those produced from unpasteurized ewes' milk, contained a richer complex of aroma-active compounds but also bacteria that may be problematic, as they were not sufficiently suppressed by treatment with hot water.

Acknowledgements

This work was supported by the Slovak Research and Development Agency under contract No. APVV-15-0006.

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Received 19 January 2019; 1st revised 8 April 2019; accepted 8 April 2019; published online 24 April 2019.