

**EFFECT OF MILK ORIGIN ON PROTEOLYSIS AND ACCUMULATION OF BIOGENIC AMINE DURING RIPENING OF DUTCH-TYPE CHEESE***Vendula Pachlová, Zuzana Charousová, Tomáš Šopík***ABSTRACT**

Dairy products from goat's milk are characterized by their distinctive aroma and their specific taste. However, the strong aroma can discourage some consumers. Properties of cheese can be modified by the combination of goat's and cow's milk. On the other hand, chemical diversity from different milk origin may affect the changes during ripening. The aim of the study was to compare the intensity of changes during ripening of model cheese samples produced with various ratios of goat's and cow's milk. The combinations 100:0 (100% goat's milk), 75:25, 50:50, 25:75 and 0:100 (100% cow's milk) were used for the manufacture of Dutch-type cheeses, which were ripened during a period of 84 days. Protein profile, free amino acid content and biogenic amine content were used for the description of cheese properties during storage. Cluster analysis showed different changes in the protein matrix of the examined samples. The results indicated that even low addition of cow's milk significantly affected the protein profile. However, the homology of protein profiles rose with the increasing ripening time. More intensive proteolysis occurred in the samples with predominance of goat's milk. Moreover, cheese samples produced only from goat's milk presented a significant increase in the amount of free amino acids after 14 days of ripening. The effect of milk origin on the production of biogenic amines was also examined. However, higher concentrations of biogenic amines were detected in samples manufactured from goat's milk. Tyramine, putrescine, histamine and phenylethylamine were detected during the storage of the samples. The total biogenic amine content exceeded 100 mg/kg in samples with predominance of goat's milk.

**Keywords:** goat's milk, cheese ripening, proteolysis, biogenic amine

**INTRODUCTION**

Dairy products from goat's milk are characterized by their distinctive aroma and their specific taste. For this phenomenon are mainly responsible fatty acids in higher concentration, such as capric acid, caprylic acid and caproic acid (Albenzio and Santilo 2011). Therefore, some consumers may find this specific strong aroma of goat's products unpleasant. Moreover, goat's milk is in comparison with cow's milk more expensive for the production of dairy products. Especially, in the case of ripened goat's cheeses, the production costs are several times higher than the cheese from cow's milk. The deficiencies mentioned above can be reduced by a combination of goat's and cow's milk. Furthermore, the flavor can be modified with the different milk composition. On the other hand, chemical diversity may affect the processes occurring during cheese ripening and thereby modify their properties. During the cheese ripening, the proteolysis is the most important process. The intensity of hydrolysis of the protein matrix of the cheese creates properties, especially a texture due to the weakening of the protein matrix (Pachlová et al., 2011).

Final products of proteolysis – free amino acids are considered as markers of ripening. These free amino acids can be subsequently converted to significant sensory active substances, while the desired flavors of ripened products are developed (Sousa et al., 2001). However, during cheese ripening decarboxylation of the free amino acids may simultaneously occur and biogenic amines concentration can be increased up to unhealthy for the consumer levels. The biogenic amines are classified as psychoactive and vasoactive substances which are responsible e.g. for the variation in blood pressure, headache, migraine, vomiting and respiratory problems. For instance, histamine, tyramine and phenylalanine contribute to these symptoms directly. Moreover, putrescine and cadaverine can act as intensifiers of toxic effects of other biogenic amines (Spano et al., 2010; Kalač, 2014).

For these reasons, the aim of the study was to describe the influence of goat's and cow's milk combinations on the properties of cheese during ripening and evaluate the content of selected biogenic amines.

## MATERIAL AND METHODOLOGY

## Samples

Model samples of Dutch-type cheese were produced in five different combinations of goat's and cow's milk 100:0 (100% goat's milk), 75:25, 50:50, 25:75 and 0:100 (100% cow's milk). Milk for cheese production was obtained from a small farm. Before the production of cheese, the milk was pasteurized (74 °C for 30 seconds). Products were wrapped in a shrink foil after salting. Model cheeses were ripened in the maturing chamber at 12 ±2 °C. Samplings were carried out on the 1, 14, 28, 56 and 84 days after production. The basic chemical analysis (dry matter content, pH and NaCl content), protein profile, free amino acid content and the content of biogenic amines were evaluated during maturation. Two parallel cheese blocks were analysed in each day of sampling.

## Basic chemical analysis

Model samples of cheese were subjected to determination of dry matter content according to **ISO 5534:2004** norm and salt content (**Indra and Mizera 1992**) and pH value (pH meter, Eutech Instruments, The Netherlands). For the lyophilization of the samples was used the ALPHA 1-4 LCS (Christ, Osterode am Harz, Germany) at -40 °C and a pressure approximately 12 Pa. The lyophilized samples were stored at -70 °C and were used to determine the protein profile, free amino acids and biogenic amines. Three parallel assessment were carried out for each samples.

## Protein profile

The preparation of samples for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure published in **Lazarkova et al. (2010)**. The electrophoretic separation was carried out by means of buffer system according to **Laemmli (1970)** and a vertical electrophoresis apparatus (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The visualisation was performed using Coomassie brilliant blue G25012 and the results were evaluated by means of Ultra Quant™ 6.0 software (Ultra-Lum. Inc., Claremont, CA, USA).

## Free amino acid content

The determination of the concentrations of free amino acids was performed by means of chromatographic analysis, which was focused on the detection of the content of 30 amino acids and their derivatives (threonine, serine, asparagic acid, asparagine, glutamic acid, glutamine, proline, glycine, alanine, citrulline, valine, cysteine, methionine, cystathionine, isoleucine, leucine, tyrosine, phenylalanine, β-alanine, β-aminobutyric acid, γ-aminobutyric acid, ethanolamine, ornithine, lysine, histidine, 1-methyl-histidine, 3-methyl-histidine, arginine, amino adipic acid, γ-aminobutyric acid). Lyophilised samples of cheeses were used to determine the content of free amino acids. The extraction of free amino acids was performed by triple extraction according to **Pachlová et al. (2011)** using Li-buffer. This compound is composed of hydrochloric acid and lithium citrate, wherein pH of Li-buffer is 2.2 ±0.2. The resulting extract was analysed by ion-exchange liquid chromatography using Automatic

Amino-Acid Analyzer AAA 400 (Ingos, Prague, Czech Republic) with a column of 150 × 3.7 mm and Polymer AAA ion exchanger according to **Buňková et al. (2009)**. Each cheese sample was extracted twice. Each extract was analysed twice.

## Biogenic amine analysis

Lyophilised cheese was used for the biogenic amine (BA) and polyamine (PA) analysis of the cheese samples. Triple extraction of BA and PA from the lyophilised samples was carried out using a perchloric acid solution (0.6 mol.L<sup>-1</sup>). Three independent extractions were performed on each cheese sample. The filtrated extract (filter porosity 0.45 μm) was then used directly for the derivatisation and determination of BA/PA content (**Dadáková et al., 2009; Buňková et al., 2013**) that followed.

The quantities present for eight biogenic amines of histamine (HIM), tyramine (TYM), phenylethylamine (PHE), tryptamine (TRY), putrescine (PUT), cadaverine (CAD), spermine (SPE) and spermidine (SPD) were analysed via liquid chromatography (LabAlliance, USA and Agilent Technologies, Agilent, Santa Clara, California, USA) after derivatisation using dansyl chloride. The dansyl chloride sample derivatisation procedure was performed according to **Dadáková et al. (2009)**. 1,7-heptandiamine was used as the internal standard. Chromatographic separation (ZORBAX Eclipse XDB-C18, 50 × 3.0 mm, 1.8 μm; Agilent Technologies) and detection (spectrophotometric λ = 254 nm) were performed according to **Buňková et al. (2013)**. Each cheese sample was analysed 12 times (3 extractions, 2 derivatisations, 2 applications to the column).

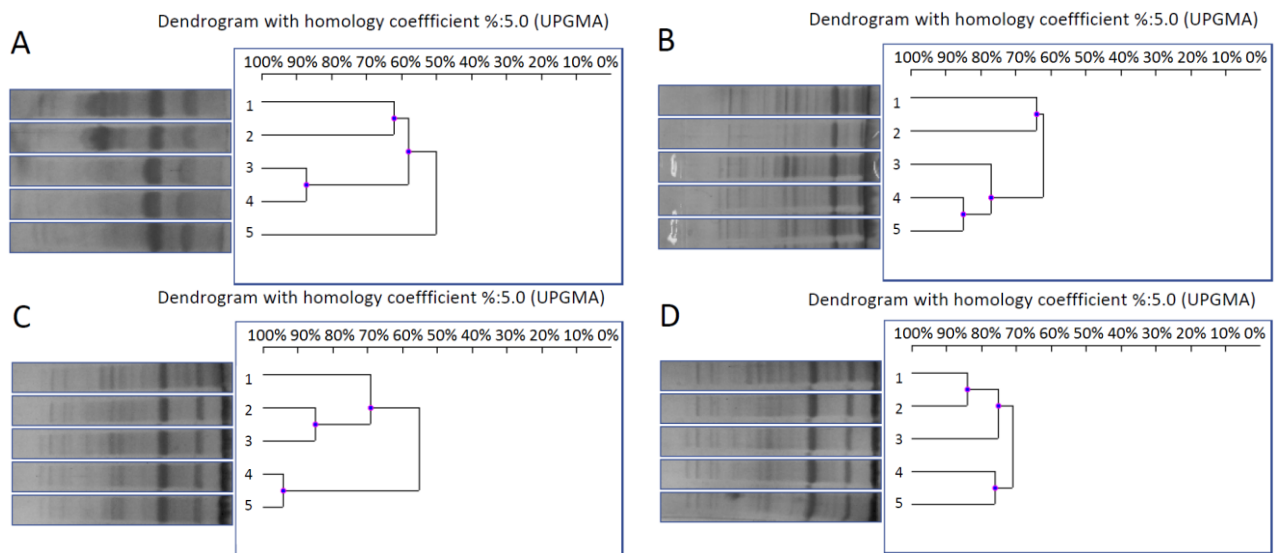
## Statistical analysis

The results of the determination of free amino acid content and content of biogenic amines were statistically evaluated by means of the Kruskal–Wallis test and Wilcoxon test. Unistat® 5.5 software (Unistat, London, UK) was used for the statistical evaluation. Cluster analysis was used to compare the results obtained by SDS-PAGE.

## RESULTS AND DISCUSSION

After production of the model samples of cheeses made in various proportions of goat's and cow's milk, the dry matter content was measured (before salting). The observed data ranged in the average of 50.3 ±0.4% (w/w). Moreover, the pH value was measured and was 4.99 ±0.05% (w/w). From the 14th day of storage, the dry matter content increased due to the salting 51.9 ±0.6% (w/w), with an average concentration of NaCl 1.45 ±0.11% (w/w). During further storage there were no significant differences in the dry matter and the salt contents between the samples (data not shown).

Cluster analysis showed significant changes in the protein matrix in samples with different proteins of goat's and cow's milk origin are illustrated in Figure 1. We observed, that the sample made only from cow's milk (sample 0:100) formed separate cluster within 24 hours after manufacturing. The results also indicated that even low addition of cow's milk at the beginning of ripening significantly affected the protein profile. Similarity around

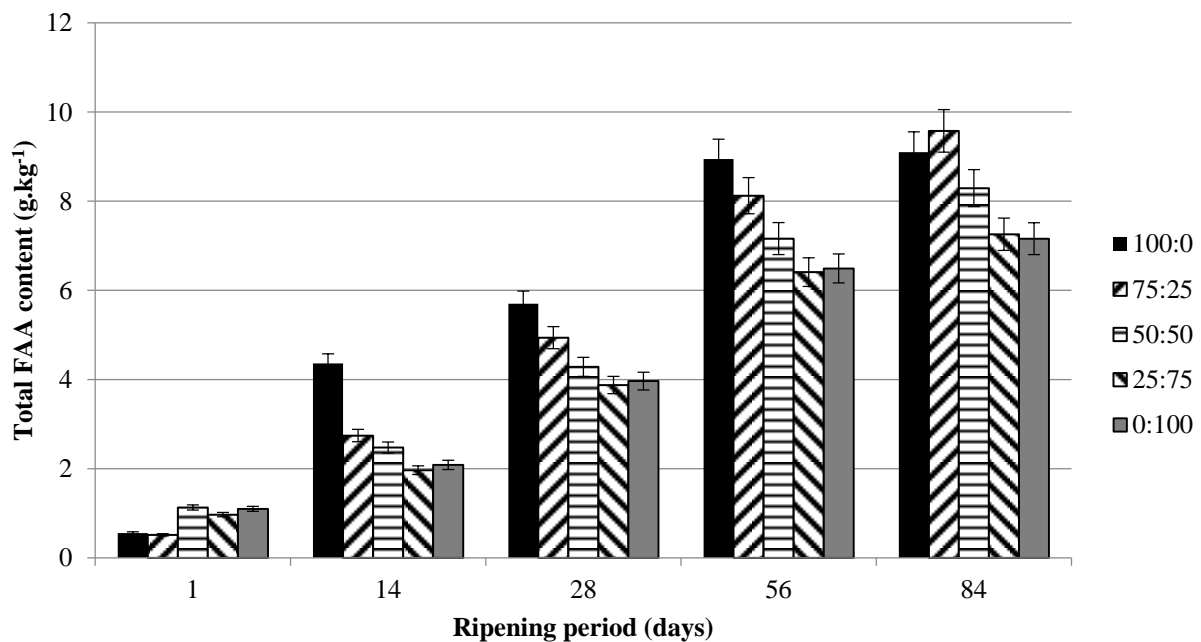


**Figure 1** Protein profile of model cheese samples with different proportion of goat's and cow's milk (1 – 100:0; 2 – 75:25; 3 – 50:50 4 – 25:75; 5 – 0:100) during ripening (Part **A** – day 1, Part **B** – day 28; Part **C** – day 56; Part **D** – day 84).

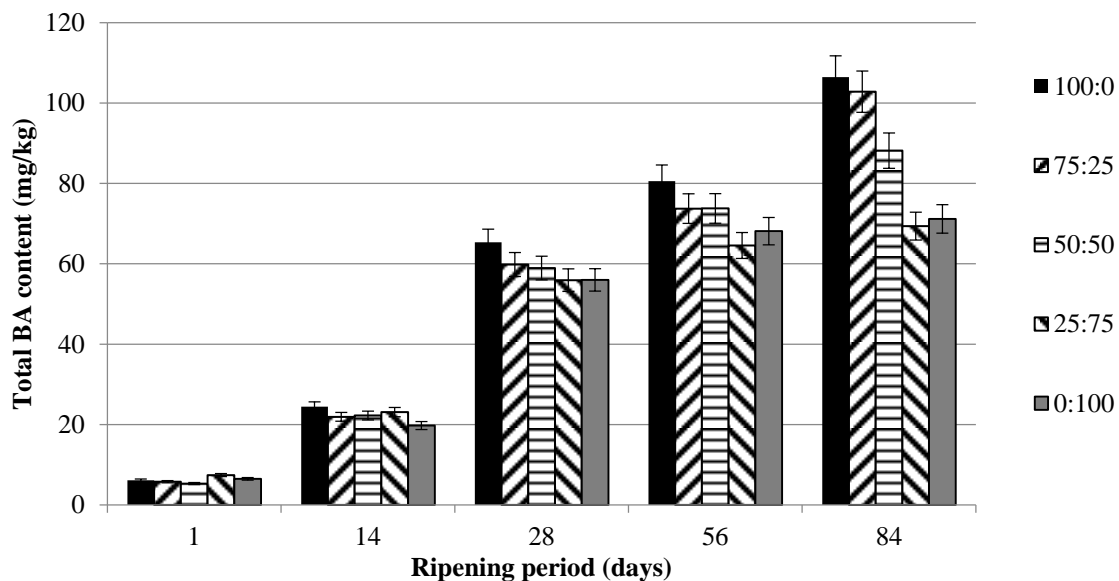
87% between the samples was observed in cheeses made of 50:50 and 25:75 ratios (goat's/cow's milk). On the other hand, samples in ratio 100:0 and 75:25 showed the lowest similarity, 62%. The homology of the samples made purely of generic milks (100:0 and 0:100) was only 53%.

After 28 days of ripening, the similarity of the samples protein profile with predominance of cow's milk began to show higher values. The protein profile of the samples 25:75 and 0:100 coincided 85% and after 56 days of ripening even reached 94% compliance. Whereas, the similarity of the sample from goat's milk (100:0) in comparison with other combined samples after 28 days of ripening decreased from 64% to 66% (in the case of sample 0:100 was demonstrated 55% homology). With the increasing time of maturation, a rise in homology was

observed. After 84 days of ripening was reported 84% similarity between samples 100:0 and 75:25. Furthermore, protein profiles of samples 0:100 and 25:75 were equal to 76%. Protein profiles of cheese samples were influenced firstly by different chemical composition of goat's and cow's milk but probably also by varied microenvironmental conditions in cheese which affect activity of microorganisms naturally present or added as starter culture. Intracellular microbial endopeptidases and exopeptidases hydrolyze protein matrix during cheese ripening while amino acids are final products of proteolysis (Fontenle et al., 2017; Sousa et al., 2001). Chemical composition of cheese can play role in intensity of proteolysis. On other hand the results showed that with the increasing maturation time the protein profile of the



**Figure 2** Total content of free amino acids in model samples of cheeses with different proportion of goat's and cow's milk during ripening.



**Figure 3** Total content of biogenic amines in model samples of cheeses with different proportion of goat's and cow's milk during ripening.

individual samples reached higher similarity.

Increase in the total content of free amino acids (Figure 2) was observed during ripening in all cheese samples. However, differences between individual batches were detected. The proteolysis was more intense in cheese with a predominance of goat's milk. Therefore, after 14 days of ripening the cheese samples made only from goat's milk contained up to twice the amount of free amino acids. This trend of higher amino acid content was observed in samples with a higher proportion of goat's milk to the end of the experiment (day 84 after manufacture). The effect of milk origin on the intensity of proteolysis was also confirmed by the development of free amino acids in cheese made from cow's milk (0:100). During the three month experiment, the lowest concentration of free amino acids was observed in cheese from cow's milk (0:100).

Through the conversion of free amino acids into sensory active substances, a variety of flavor and aroma compounds are developed in the ripened cheeses. The intensity of proteolysis and subsequently development of volatile compounds may be influenced by both external (e.g. temperature and aging time) and internal factors (e.g. activity of microorganisms, presence and activity of endogenous and exogenous enzymes, water activity, pH) (Bezzera et al., 2017; Combarros-Fuertes et al., 2016; Hickey et al., 2013). In terms of representation of endogenous enzymes milk of different species vary significantly. For instance, the goat's milk contains in comparison with cow's milk an increased representation of proteinase enzymes (Albenzio and Shantilal 2011). The activity of the present enzyme can be further regulated, inter alia the availability of substrate for specific enzymes (Sousa et al., 2001). These factors significantly affect the formation of long polypeptide chains differently and, ultimately, the content of free amino acids which can be converted into sensory active compounds.

The content of biogenic amines such as tyramine, putrescine, histamine, and phenylethylamine were monitored during storage. The results showed an increase in biogenic amine concentration depending on

the time of maturation in all batches of model samples. During the first 14 days of ripening, the no significant differences between the samples were observed. After 28 days was observed a higher concentration of the sum of biogenic amines in the samples produced from the goat's milk in comparison with other samples as can be seen in Figure 3. Moreover, this trend was continuous until the end of the experiment. Higher content of biogenic amines in goat's cheese was detected also in Buňková et al. (2013).

During 84 days of ripening was observed an increase in the content of biogenic amines above  $100 \text{ mg} \cdot \text{kg}^{-1}$  in the samples with a predominance of goat's milk (samples 100:0 and 75:0). Such high concentrations can be evaluated as a risk for the consumer, as they can cause unwanted psychoactive and vasoactive effects (Ten Brink et al., 1990; Silla Santos 1996).

Increased accumulation of biogenic amines in the samples with a predominance of goat's milk was attributable due to several factors. Firstly, the increased proteolysis and consequently preferable availability of amino acids as precursors for the formation of biogenic amines. Secondly, the raw milk was a probable source of microorganisms capable of producing biogenic amines in ripened cheeses (Combarros-Fuertes et al., 2016). Additionally, raw goat's and cow's milk may differ in microflora. Moreover, even though milk was subjected to heat treatment, some groups of microorganisms may survive pasteurisation and subsequently contribute to the changes during maturation (Quigley et al., 2013; Kološta et al., 2014).

## CONCLUSION

Based on the results of the study it can be concluded, that the type of milk used has a significant influence on the intensity of changes during ripening. The differences in the intensity of proteolysis and biogenic amine content were observed in samples from goat's and cow's milk. However, with the increasing ripening period, the differences in protein profiles decreased. The most intense

proteolysis was observed in samples with a predominance of goat's milk. Moreover, with the rising content of cow's milk, the deceleration of proteolysis was observed. Higher concentrations of biogenic amines were detected in model cheese manufactured from predominant goat's milk.

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