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THE EFFECT OF REDUCTION OF NaCl CONTENT ON SELECTED PARAMETERS DURING RIPENING OF CHEESE

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ABSTRACT
The aim of this work was to observe chemical and physical changes in Dutch-type cheese during ripening depending on salt concentration. Ripening is one of the most important factors influencing the sensory quality of cheese and therefore the cheese production should be studied. Among the substances which are formed during ripening belong the biogenic amines which are produced by the decarboxylation of amino acids. These amino acids are created during proteolysis. The salt content largely affects the intensity of the ripening process, but also other cheese parameters such as dry matter content, hardness or content of biogenic amines. In the course of 3 months ripening of model cheeses with different cultures and with different salt content, the effect of the salt on pH, dry matter content, free amino acids and biogenic amines content and hardness was monitored. The concentration of NaCl affected the dry matter content and the hardness of the samples. The reduction in salt content contributes to the higher accumulation of biogenic amines during ripening.

Keywords: cheese; cheese ripening; proteolysis; biogenic amines; salt content

INTRODUCTION
Approximately one third of world milk production is used in cheese production. Cheese is a high nutritional food. In addition to other parameters, the types of cheeses also differ in salt content (NaCl) **(Farkye, 2004)**. In the case of Dutch-type cheese the salt content is between 1.5 – 3% **(Guinee, 2004)**. The salt in the cheese serves as a preservative, which contributes to the sensory properties of the cheese. Salt also serves as a source of sodium, which is important for the consumer in terms of regulation of blood pressure, water transport into and out of the cells, osmolality of tissues and cellular pulse nerve transfer **(Guinee, 2004)**. In addition to the above mentioned effects, salt content affects microbial growth, enzymatic activity or biochemical changes in cheese ripening, which affect their sensory properties **(Guinee, 2004; Shalaby, 1996)**. The current trend in food production is to find ways to reduce salt in food (reformulation) because of its high intake to the consumer and the associated health impact most often to increase blood pressure **(Guinee, 2004)**. Lowering the salt concentration, however, could affect the microenvironment, which may be more beneficial for the development of undesirable microflora. One of the undesirable manifestations of activity contaminating microflora is the production of biogenic amines. These compounds may, in case of higher intake, have an adverse effect on the health of the consumer. The most well-known effects of biogenic amines on the health of the consumer are their negative influence on the nervous and cardiovascular system **(Shalaby, 1996)**.

The aim of the work was to investigate the effect of reduction of salt content on textural properties, intensity of proteolysis and accumulation of biogenic amines in Dutch-type model cheeses during 3 months ripening.

Scientific hypothesis
Decreasing the concentration of NaCl promotes the activity of BA-producing microorganisms.

MATERIAL AND METHODOLOGY
Production of model samples
Model samples of cheese were made **(Pachlová et al., 2011)**. First, raw milk was preheated to 35 °C, followed by centrifugation of milk (Disc Bowl Centrifuge FT15, Armfield Inc., UK) and the standardization of the fat content of 2.5%. Pasteurisation with FT75 laboratory pasteuriser (Armfield Inc., UK) was performed (74 °C for 30 seconds). After pasteurisation, the milk temperature was adjusted to an inoculation temperature of 32 °C. For the production of a model batch of cheeses, 0.5 g of mesophilic culture of Flora Danica (samples marked as FD) or CHN19 (Chr. Hansen, Denmark) was added to the 20 litres of pasteurized milk. Subsequently, 10 mL of $CaCl_2$ (36% solution, Milcom a.s., Czech Republic) were applied. Culture was activated for 30 minutes. Coagulant (640 μL, Chymax M 1000, Chr. Hansen, Denmark) was used for renneting (30 minutes at 32 °C). After this process, the precipitate was carefully cut and left for 10 minutes to stand for curing. Curd was gentle stirred for

20 minutes. Before the cooking, 5 litres of whey were collected and the water at 80 °C was added to reach a temperature of 42 °C. Curd was stirred at 42 °C for 90 minutes. After this process, the curd was moved into molds where was pressed for 90 minutes. Pressed cheeses were left at 16 °C overnight. Each model batch was divided into 2 groups of cheese blocks. The first part was brined to concentration of 1.5% NaCl (samples marked as LS) and the second part of cheese blocks to 2.5% NaCl (samples marked as MS). NaCl content of brine was 20%. After brining, Delvocid (DSM, Netherlands) was applied on the surface of cheese blocks. The model cheese blocks were wrapped in a shrink foil and stored in a ripening chamber at a temperature of 12 ±1 °C. Samples of model cheeses were taken for analysis after the 14th, 28th, 56th and 84th day of ripening. Two blocks of model cheese were used for sampling from each batch. The production of cheeses with the selected culture was repeated twice.

Basic chemical analysis

The basic chemical analysis was focused on determining the dry matter content at a temperature of 102 ±2 °C (**ISO 5534:2004)**, NaCl content and value of pH according to **Flasarová et al. (2016)**. The samples were subsequently lyophilised **(Pachlová et al., 2011)** for determination of the free amino acid and biogenic amine contents.

Textural profile analysis

The texture evaluation was focused on monitoring the hardness of cheeses using TA.XT Plus (Stable Micro Systems, UK). A central cylinder of 35 mm in diameter and 20 mm in height was cut out of the cheese sample. The texture of the semi-hard cheese was evaluated by a 50 mm diameter probe by compression test. The sample was compressed by 25% of the original height at 2 $mm.s^{-1}$. The measurement was carried out at room temperature (20 ±2 °C).

Determination of free amino acids

The lyophilised samples of the cheeses were used to determine the free amino acid content. Total free amino acid content was expressed as sum of the concentrations of 30 free amino acids and their derivates (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-methylhistidine, 3-methyl-histidine, arginine, aminoadipic acid, α-aminobutyric acid). The extraction of the free amino acids was performed by triple extraction using Li-buffer according to **Pachlová et al. (2011)**. The resulting extract was analysed by ion-exchange liquid chromatography using Automatic Amino-Acid Analyzer AAA 400 (Ingos, Prague, Czech Republic) according to **Flasarová et al. (2016)** and **Buňková et al. (2009)**. Two blocks of cheese from each group were sampled and each extract was subjected to the chromatographic analysis twice (2 repetitions of manufacture × 2 cheese block × 2 extractions × 2 separation and determination of eluents; n = 16).

Determination of biogenic amines

The lyophilised cheese samples were used to determine the biogenic amine content (histamine, tyramine, phenylethylamine, tryptamine, putrescine, cadaverine, spermidine and spermine). The triple extraction from the lyophilised cheeses was performed using 0.6 $mol.L^{-1}$ $HClO_4$ (Merck, New Jersey, USA) according to **Flasarová et al. (2016)** and **Dadáková, Křížek and Pelikánová (2009)**. After this process, the detection and separation of the biogenic amines was performed by means of high performance liquid chromatography (Agilent Technologies, Santa Clara, USA) using Agilent Eclipse Plus C18 RRHD column (Agilent Technologies, Santa Clara, USA) with dimensions of 3.0 × 50 mm × 1.8 μm and spectrophotometric detection at a wavelength of 254 nm and a column temperature of 30 °C **(Flasarová et al., 2016; Smělá et al., 2004)**. The extraction of biogenic amines was performed twice and each extract was subjected to chromatographic analysis twice (2 repetitions of manufacture × 2 cheese block × 2 extractions × 2 separation and determination of eluents; n = 16).

Statisic 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.

RESULTS AND DISCUSSION

Basic chemical analysis

After 14 days of ripening, the pH of samples inoculated with CHN19 culture was 4.91 – 5.01. Subsequently, the pH grew to 5.32 – 5.51 after 56 days of ripening. At the last sampling (84th day of ripening), a slight decrease was observed to values ranging from 5.28 – 5.38. There were no significant differences between samples with different NaCl concentration ($p \geq 0.05$). The average development of pH in FD-inoculated blocks after 14 days of ripening was 5.41. Subsequently, the pH slightly decreased to 5.35 – 5.38 (28th day of ripening). After 56 days of ripening, the pH rose to 5.46 and after 84 days of ripening reached values of 5.30 – 5.47. Differences between individual groups of model samples could arise due to the varying intensity of lactic acid production used by the culture. Non-starter lactic acid bacteria are also involved in pH change and during the ripening gradually prevail over the starter cultures and significantly contributed to the changes in matrix of ripening cheese **(Rynne et al., 2007)**.

The dry matter content slightly fluctuated during ripening. From the values found, in the case of MS cheeses (2.5% NaCl), the values on average of 2% of the dry matter content were higher than those for LS cheeses (1.5% NaCl). The dry matter content of MS samples ranged around 58.4%, while the dry matter content of LS samples was around 56.4%.

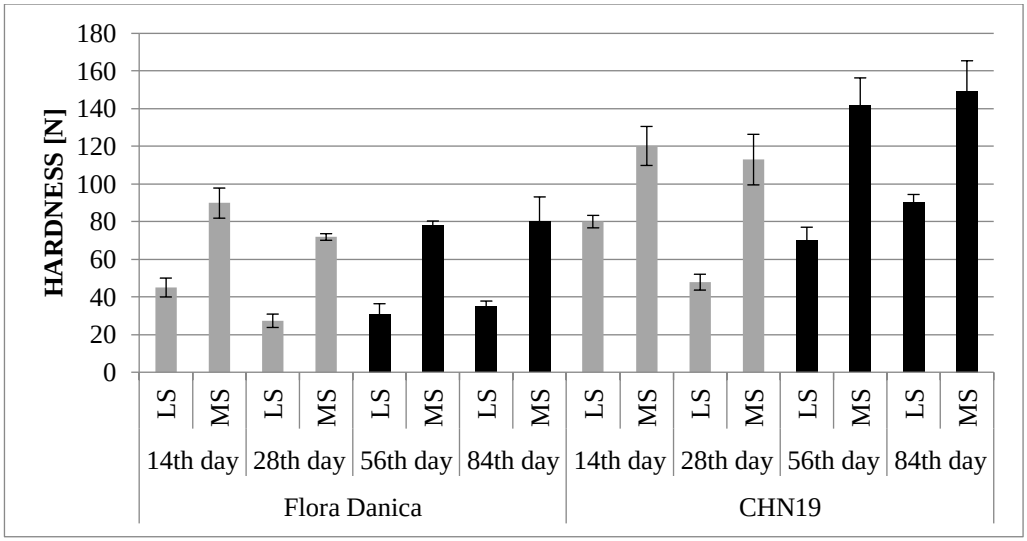

Figure 1 The development of hardness during ripening of model cheese samples: LS – model cheese with 1.5% NaCl content; MS – model cheese with 2.5% NaCl content.

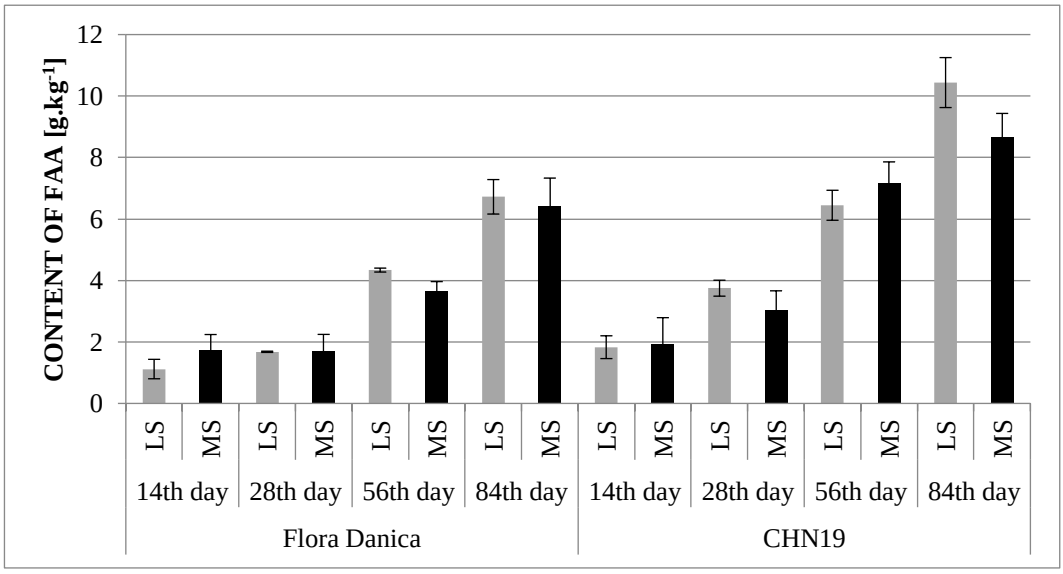

Figure 2 Free amino acid content during ripening of model cheese samples: LS – model cheese with 1.5% NaCl content; MS – model cheese with 2.5% NaCl content.

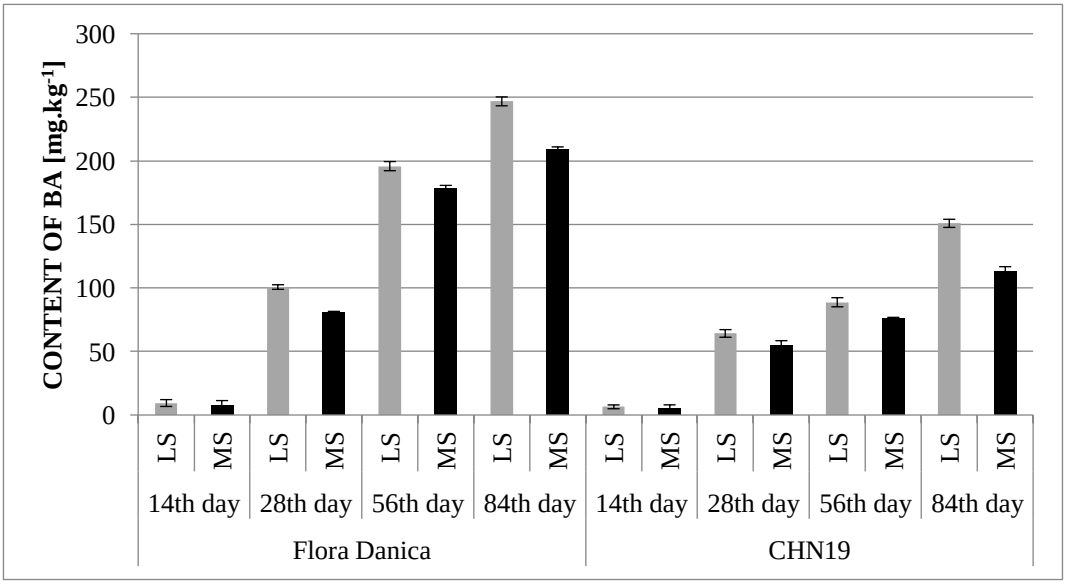

Figure 3 Biogenic amine content during ripening of model cheese samples: LS – model cheese with 1.5% NaCl content; MS – model cheese with 2.5% NaCl content.

Textural profile analysis

The effect of culture on hardness values was observed (Figure 1). In general, samples with FD culture have reached lower hardness values compared to CHN19 culture samples. After the initial decrease in hardness, a gradual increase in hardness was observed in all samples. A more pronounced trend was observed with samples of CHN19 culture. The hardness of the cheese depended mainly on the salt content and its associated dry matter content, where samples with higher NaCl concentration had a higher dry matter content and a higher hardness (Figure 1). In the case of FD-MS model cheeses, hardness was higher more than twice compared with FD-LS samples after 84 days of ripening. A similar trend was also observed for CHN19 samples. Higher hardness in the case of cheese with higher NaCl concentration was also found in Kaya's research **(Kaya, 2002)**.

Determination of free amino acids

The total content of free amino acids (FAA) in cheese blocks (Figure 2) increased during ripening as a result of proteolysis of the casein matrix **(Visser, 1993)**. In general, the free amino acid content of the MS samples was lower regardless of the culture used. This trend can be consequence of a reduction in the activity of the microflora and its proteolytic enzymes by higher salt concentration. **Murtaza et al. (2014)** also observed lower FAA contents in higher salt samples. In the case of MS cheeses, there was a limited trend in amino acid release until 28 days after production, which may be due to a slower diffusion of salt into the central parts of the blocks. Initial proteolysis is furthermore initiated by chymosin and plasmin, whose activity is not dependent on the salt content, whereas the salt content has an effect on the activity and growth of lactic acid bacteria **(Exterkate and Alting, 1995)**. In addition, different total FAA contents were determined in samples of different cultures. Higher concentrations of FAA were observed in CHN19 culture samples from 28 days of ripening. Different concentrations of FAA in model cheese with different cultures may be due to both the different proteolytic activity of the microflora present, but also by the decarboxylation of amino acids to biogenic amines **(Halász et al., 1994; Butor et al., 2017)**, as indicated by the results of the total concentration of biogenic amines (see Figure 3).

On the other hand, after 56 days of ripening the FAA content of CHN19-MS samples increased sharply. Compared with other sampling days, these FAA values are in contradiction with the trend of FAA concentrations which were determined in model cheeses with a lower salt content. The rationale can be found in extensive lysis of cells in environments with higher salt concentrations. In the case of FD-type cheeses, however, this phenomenon was not observed.

Determination of biogenic amines

The content of biogenic amines (BA) in the samples (Figure 3) increased with increasing ripening. The content of BA in the samples increases with the degree of proteolysis of the casein network, respectively by releasing their precursors – free amino acids **(Halász et al., 1994)**. The second observed trend is related to salt content in cheese blocks, where a lower total content of BA was observed in samples with higher salt concentrations. After 14 days of ripening, the content of BA in all samples was relatively low and ranged from 5.32 – 12.23 mg.kg^{-1}. After the 28th day of ripening, the BA content has increased sharply in all batches, probably as a result of the growth and activity of non-starter lactic acid bacteria **(Bover-Cid et al., 2001)**, which are referred to in many publications as the cause of the accumulation of BA during ripening of cheeses **(Shalaby, 1996; Stratton, Hutkins and Taylor, 1991; Leuschner, Kurihara and Hammes, 1999)**. For FD-MS model cheeses, the total content of BA was nearly 20% lower after 3 months ripening compared to LS samples. In the case of CHN19 cheeses, even the BA accumulation rate for MS cheeses was lower by more than 25%. This can be explained by partial inhibition of proteolysis but in particular by suppression of decarboxylase activity of bacteria due to increased NaCl content. From the established values it can be stated that the reduction in salt content may contribute to the higher accumulation of BA during ripening of the FD-MS cheese and **Gardini et al. (2001)** also measured similar results.

CONCLUSION

From the results obtained it can be stated that the salt content has a significant influence (p <0.05) on the course of biochemical processes during maturation of the cheeses. Higher concentration of salt caused the reduction of microbial activity. Subsequently lower total FAA content was determined in cheese with higher salt content. Similarly lower total content of BA was observed in samples with higher salt concentration. BA content in model cheese with 2.5% NaCl content was 20% lower after 3 months ripening copared to model cheese with 1.5% NaCl content. It has been also observed that higher concentration of NaCl increased the dry matter content, which is reflected in the higher hardness of the cheese blocks. No unambiguous trend was observed between pH values in samples with different salt contents.

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