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REGULAR ARTICLE

CHANGES OF BIOGENIC AMINE CONTENT AND OTHER SELECTED PARAMETRES IN WHITE CHEESE MODEL MATRIX

Vendula Pachlová[*1], Richardos-Nikolaos Salek[1], František Buňka[1], Leona Buňková[2]*

Address: [1] Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlín, nám. T. G. Masaryka 5555, 760 01 Zlín, Czech Republic.

[2] Department of Environmental Protection Engineering, Faculty of Technology, Tomas Bata University in Zlín, nám. T. G. Masaryka 5555, 760 01 Zlín, Czech Republic.

*Corresponding author: pachlova@ft.utb.cz

ABSTRACT

The objective of our study was to compare the effect of addition of decarboxylase positive strain of *Lactococcus lactis* subsp. *lactis* on biogenic amine production, intensity of proteolysis and changes of hardness during ripening of white brined cheese. Model samples were kept under 10 °C for 56 days. The FAA content of cheese and hardness of samples were increasing during ripening. Significant differences in FAA content and also hardness were not found between control samples and samples with decarboxylase positive lactococci ($P \geq 0.05$). Production of biogenic amine was more intense in sample with addition of decarboxylase positive lactococci (especially during first 28 days of ripening) and depends on initial micro flora during first stage of ripening. However, environmental conditions and ripening time are important factors which play major role in biogenic amine production through the microorganism growth in following ripening phases.

Keywords: biogenic amine, cheese ripening, decarboxylase activity

INTRODUCTION

White brined cheeses are the group of cheese varieties ripened and preserved in brine for a considerable amount of time, i.e. until consumption (**Alichanidis and Polychroniadou, 2008; Moatsou and Govaris, 2011**). The ripening period depends on the cheese variety. White brined cheeses usually ripen from a few weeks to several months (**Fox *et al.*, 2004a**). Ripening includes microbiological and biochemical changes which affect organoleptic and texture properties of the cheese (**Pachlova *et al.*, 2012**). Proteolysis is very important part of cheese ripening. The principal role of the proteolysis is the liberation of free amino acids (FAA) as precursors for a complex series of catabolic reactions to give flavour compounds (**Fox *et al.*, 2004b, Bontinis *et al.*, 2011**). On the other hand, biogenic amine can be formed by microbial decarboxylation from free amino acids or by amination and transamination of aldehydes and ketones (**Silla-Santos, 1996**) during cheese ripening. Consumption of food containing high concentrations of biogenic amines (>100 mg/kg) could cause toxic or some deleterious effects. Many studies deal with the occurrence of biogenic amines in various foods (especially fermented) such as wine, beer and also cheese (**Kalač *et al.*, 2002; Cortacero-Ramírez *et al.*, 2007, Komprda *et al.*, 2008, Buňková *et al.*, 2010)**. Other studies such as **Lorencová *et al.* (2012)** or **Buňková *et al.* (2012)** deal with selection and study of microorganism (such as lactic acid bacteria), which are major producer of biogenic amine. However, these works explore the particular biogenic amine production in growth medium, where the concentration of biogenic amine could be biased optimal environment for the bacteria metabolisms. Moreover, some strains of the starter lactic acid bacteria (such as *Lactococcus lactis* subsp. *lactis.*) have decarboxylase activity that was observed in model environment of growth broth. Behaviour of these strains has not been investigated in real system of the cheese and can be different in comparison with condition in growth broth.

The objective of our pilot study was compare the biogenic amine content and other selected parameters in control cheese and cheese, which were manufactured with decarboxylase positive strain of *Lactococcus lactis* subsp. *lactis.*

MATERIAL AND METHODS

Cheese manufacturing

Two parallel batch of cheese ripened in brine were manufactured under the same laboratory conditions (first control batch, second batch with decarboxylase positive culture addition). Each batch of cheese was produced from 4 l of pasteurized cow's milk (fat content 2.5 %). Milk was tempered to 34 °C. Yoghurt and mesophilic cultures were added in ratio 1:1 (2 % of whole milk volume; mesophilic culture: *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* var. *diacetilactis*; yoghurt culture: *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*; Laktoflora®, Milcom a.s., Czech Republic). Both of the above cultures were tested for decarboxylase activity. Cultures did not produce biogenic amines. Next $CaCl_2$ solution (2 mL of 37 % w/v, Milcom a.s., Czech Republic) was added to milk for better renneting. After 30 minutes, 0.274 mL of rennet was added to milk (750 IMCU, Fromase 750 TL, DSM Food Specialties). In case of second batch, 5 mL of decarboxylase positive *Lactococcus lactis* subsp. *lactis* culture was inoculated in milk immediately before addition of rennet. Amount of inoculated microorganisms was approx. $2.5 \cdot 10^8$ CFU·mL^{-1}. Coagulation time was 50 minutes. The curd was cut cross-wise into cubes (2×2×2cm). Then the curd was stirred for 50 minutes. Then curd was transferred to special plastic moulds for whey exudation and shape formation. Cheese blocks were left overnight under constant temperature (20±2 °C) and relative humidity conditions. The next day, the cheese (pH of curd 4.9) was placed into brine with salt concentration 18 % w/v. The ripening period was 56 days under 10±2 °C). Cheese sampling was in 1st, 28th and 56th day from manufacturing. Experiment was performed three times.

Basic chemical analysis

A calibrated pH-meter (pH Spear for food testing, Eutech Instruments) was used to measure pH of the samples. Dry matter content was determined by gravimetric method according to **ISO 5534 (2004)**. Sodium chloride content was determined by argentometric method according to **Indra and Mizera (1992)**. All reagents used were of analytical grade (LachNer, Czech Republic). All parameters of each type of sample and each layer were measured six times.

Determination of free amino acid content

Prior to the analysis of free amino acids content, the samples of individual batches were lyophilized using a Christ Alpha 1–4 (Christ, Germany) device and then they were stored at -80 °C. Lyophilized samples were subjected to extraction as reported by **Pachlová *et al.* (2011)**. The free amino acid content were determined by ion-exchange chromatography (AAA400 Amino Acid Analyser; Ingos, Prague, Czech Republic) as reported by **Buňková *et al.* (2009)**. Each extract was analysed twice. The reagents for preparation, separation, and analysis of samples were obtained from Ingos (Prague, Czech Republic). The standards were purchased from Sigma Aldrich (St. Louis, MO, USA).

Determination of biogenic amine content

Prior to the analysis of free amino acids content, the samples of individual batches were lyophilized using a Christ Alpha 1–4 (Christ, Germany) device and then they were stored at -80 °C. Contents of eight biogenic amine (tryptamine, TRY; phenylethylamine, PHE; putrescine, PUT; cadaverine, CAD; histamine, HIS; tyramine, TYR; spermine, SPN; and spermidine, SPD) were determined after precolumn derivatisation with dansylchloride. Dansylchloride and BA standards were obtained from Sigma-Aldrich. Lyophilized samples were extracted with perchloric acid (0.6 M, Sigma-Aldrich) accordind to **Lorencová *et al.* (2012)**. The prepared sample was subjected to derivatisation according to **Dadáková *et al.* (2009)**. The sample was filtrated through a filter with porosity 0.22 μm and applied on a column (Zorbax Eclipse XDB-C18, 4.6x150mm, 3.5 um, Agilent technologies) of a chromatography system (binary pump and autosampler LabAlliance, USA; UV/VIS-DAD detector (λ = 254 nm), degasser 1260 Infinity and column thermostat, Agilent Technologies, Agilent, Paolo Alto, CA, USA) was used for the determination of biogenic amines.

Texture analysis

Texture analysis was performed using a TA.XTplus Texture Analyser (with compression cell in capacity of 30 kg) (Stable Micro Systems, Surrey, UK). Hardness as mechanical textural attribute relating to the force required to achieve a given deformation was observed. A cylindrical probe (P/50) was used for compression of the cheese samples at the speed of 1 mm/s. The software used for sample analysis and evaluation was the Exponent Lite software.

RESULTS AND DISCUSSION

Dry matter content of cheese samples was 40.35±0.28 % in 1st day from manufacturing and increased to 48.02±0.60 % after 56 days of ripening regardless of the decarboxylase positive lactococci addition (P≥0.05). Significant increase in the dry matter content was probably due to diffusion of salt from brine. Salt content was 8.85±0.60 % after 56 days of ripening. Also the analysis of the pH values of all the manufacture series (control cheese and cheese with the addition of the decarboxylase positive lactococci) presented the same trend during the whole experimental period (56 days). Value of pH was 4.69±0.24 in 1st day from manufacturing and increase to 4.81±0.27 during 28 days of ripening under constant temperature and moisture conditions. Values of pH dropped to 4.51±0.21 to the end of experiment (56th day of ripening). Reduction of pH values was probably due to higher activity of acidifying NSLAB.

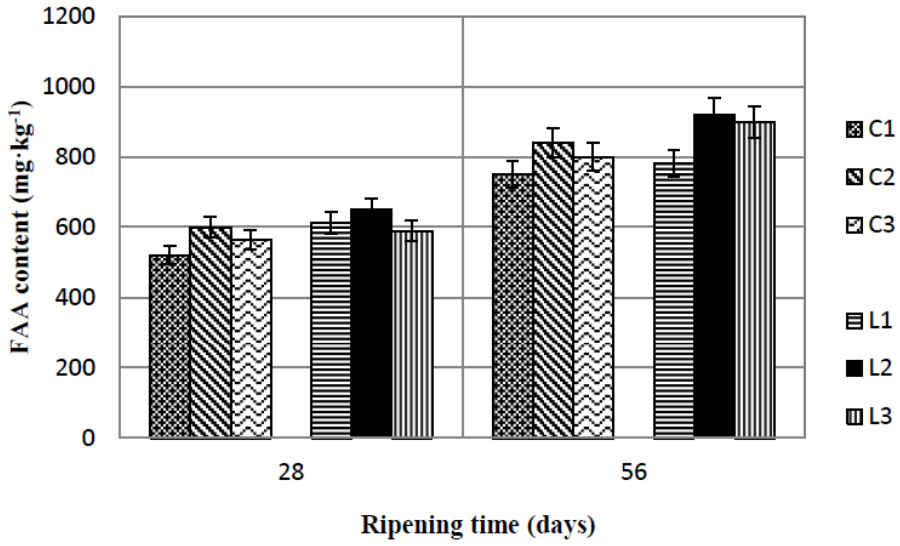

Figure 1 Total free amino acid content of cheese ripened in brine at 28th and 56th day of ripening: C1, C2 and C3 control samples; L1, L2 and L3 samples with decarboxylase positive *Lactococcus lactis* subsp. *lactis*

The results of the FAA content of manufactured cheese samples presented the same increasing trend (Fig. 1). Significant differences were not found between control samples and samples with decarboxylase positive lactococci (P>0.05) although slightly higher proteolysis was observed in cheese with addition of decarboxylase positive lactococci. Free amino acid release is attributed to the action of microbial peptidases. Aminopeptidases of the starter microorganisms (mesophilic and yoghurt cultures) were probably responsible for the massive

production of free amino acids during the first 28 days of ripening. Lactococcal peptidases are intracellular and their extensive action indicates cell lysis. According to **Valsamaki *et al.*, 2000**, the very high salt content and the low pH of the curd may create favourable conditions for cell lysis.

Biogenic amine contents of the control cheese showed that the major biogenic amines were putrescine, tyramine, spermidine and spermine. Histamine, one of the most commonly implicated biogenic amine with food poisoning (**Silla Santos, 1996, Valsamaki *et al.*, 2000, Pachlova *et al.*, 2012**), was not determined in the cheese samples. Phenylethylamine, tryptamine, cadaverine were not detected at the control cheese at 28 days of ripening in brine. The absence of cadaverine may be explained either by a lack of strains with lysine-decarboxylating activity or most probably by the inhibition of this activity by the unfavourable environment of white brined cheeses (**Valsamaki *et al.*, 2000**). At the cheese with decarboxylase positive lactococci, a small amount of phenylethylamine (4.6 $mg \cdot kg^{-1}$) as detected in 28th day of ripening.

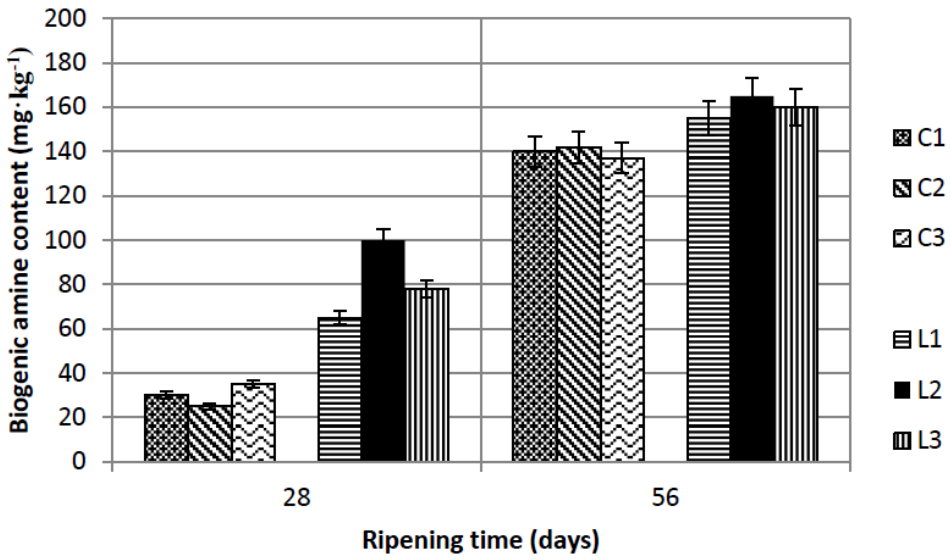

Figure 2 Total biogenic amine content of cheese ripened in brine at 28th and 56th day of ripening: C1, C2 and C3 control samples; L1, L2 and L3 samples with decarboxylase positive *Lactococcus lactis* subsp. l*actis*

Generally, the total biogenic amine content significant increased from 28th day to 56th day of ripening under constant temperature and moisture conditions (Fig. 2). At the 28th day of ripening the total biogenic amine content of cheese manufactured with the addition of decarboxylase lactococci was significantly higher in comparison with the control cheese ($P \leq 0.05$). The highest amount was observed in case of putrescine in all samples. During next

ripening (56th day from manufacturing) total biogenic amine content of control cheese significant increased to over 140 mg·kg^{-1} and was similar to the total biogenic content of cheese manufactured with decarboxylase positive lactococci. Production of biogenic amine is affected by initial micro flora in first stage of ripening. On the other hand, environmental conditions and ripening time are important factors which play major role in microorganism growth and also biogenic amine production in following ripening phases. Moreover increasing amount of biogenic amine content is not entirely dependent only on initial micro flora especially during extended ripening. Individual microbial species and strains could vary during cheese maturation while differences in the composition are mainly influenced by environmental conditions and the rate of cell lysis.

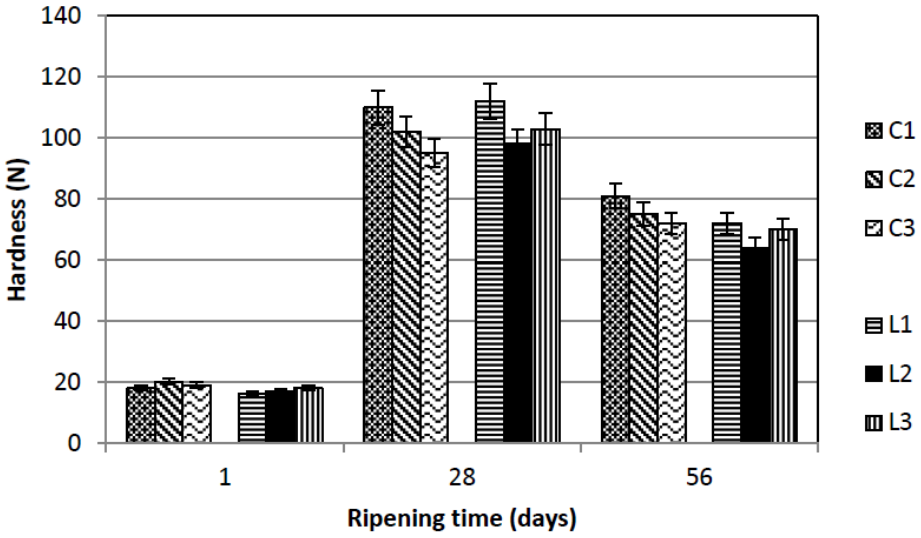

Figure 3 Development of hardness during 56-days ripening of cheese ripened in brine: C1, C2 and C3 control samples; L1, L2 and L3 samples with decarboxylase positive *Lactococcus lactis* subsp. *lactis*

The changes of hardness of the control cheese and cheese manufactured with the decarboxylase positive lactococci addition during the ripening period (56 days) is shown at Fig. 3. Texture analysis monitored in the control cheese and cheese with the decarboxylase positive lactococci addition revealed a similar trend. Hardness increased from 1st day of ripening to 28th day. On the contrary, hardness decreased from 28th day. Soft texture of manufactured cheese was probably due to the higher moisture content than in the case ripened cheese (**Raphaelides *et al.*, 1995**). During the first 28 days of ripening, all samples became more firm. One of the possible explanations can be found in the moisture loss which forms the

structure more compact. After 56 days of ripening, the texture of all samples became progressively softer probably due to proteolysis (**Raphaelides *et al.*, 1995**). From comparison of results, the changes of hardness were a little bit more intensive at the cheeses with lactococci addition ($P \geq 0.05$). It could be due to different composition of microorganisms and their proteolysis activity in the control samples and samples with the decarboxylase positive lactococci addition.

CONCLUSION

Significant differences of FAA content were not found between control samples and samples with decarboxylase positive lactococci ($P > 0.05$) although slightly higher proteolysis was observed in cheese with addition of decarboxylase positive lactococci. The changes of hardness were a little bit more intensive at the cheeses with lactococci addition ($P \geq 0.05$). It could be due to different composition of microorganisms and their proteolysis activity in the control samples and samples with the decarboxylase positive lactococci addition. At the 28^{th} day of ripening the total biogenic amine content of cheese manufactured with the addition of decarboxylase lactococci was significantly higher in comparison with the control cheese ($P \leq 0.05$). During next ripening (56^{th} day from manufacturing) total biogenic amine content of control cheese significant increased to over 140 $mg \cdot kg^{-1}$ and was similar to the total biogenic content of cheese manufactured with decarboxylase positive lactococci. Production of biogenic amine is affected by initial micro flora in first stage of ripening. On the other hand, individual microbial species and strains could vary during cheese maturation and could contribute to the different intensity biogenic amine production.

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