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# BIOGENIC AMINES IN SMEAR RIPENED CHEESES

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## ABSTRACT
Cheeses belong to high protein foods in which enzymatic and microbial activities form amino acids, which are then converted into biogenic amines (BAs) by the activity of bacterial decarboxylases. The most important conditions for BA formation include the presence of microorganisms, the availability of substrate, temperature and storage period, water activity, salt concentration, and the hygiene of the manufacturing process. Tyramine, histamine, 2-phenylethylamine, tryptamine, cadaverine, putrescine, spermidine and spermine were detected in smear ripened cheeses stored in different temperature regimes. The highest ($p$ <0.05) total BA content was found when storing the cheeses at the end of BBD (best before date) after 35 days in storage regime (A) or (C). During storage in regime (B), the total BA content ($p$ <0.05) after 49 days of storage was higher than on the production date (B/0). During storage, the tyramine content in regime (B) did not change ($p$ >0.05), while in the temperature regimes (A) and (C), the highest levels of tyramine and putrescine content were recorded in cheeses at the end of BBD after 35 days ripening. The content of polyamines in cheeses was higher ($p$ <0.05) at the end of storage than at the beginning, in all temperature regimes.

**Keywords:** biogenic amines; polyamines; smear ripened cheese;storage temperature and period

## INTRODUCTION
Cheeses are food that is often associated with the content of biogenic amines (Bas) **(Poveda, Molina and Gómez-Alonso, 2016)**. During ripening, substantial changes in the composition of cheeses take place **(Pinho et al., 2004)**. Protein degradation leads to accumulation of free amino acids, which are then converted into BAs by bacterial decarboxylases **(Komprda et al., 2007)**. Contaminant microorganisms such as enterococci, Enterobacteriaceae and lactic acid bacteria, such as lactobacilli **(Madejska, Michalski and Osek, 2017)**, contribute to the formation of BA.

Among the most important BAs found in foods are tyramine, histamine, cadaverine, 2-phenylethylamine, tryptamine, putrescine, spermine, and spermidine **(Önal, Tekkeli and Önal, 2013)**. Smear ripened cheeses have a higher BA content compared with other types of cheeses, which is related to the high protein content, extensive proteolysis, and highly active microbes with decarboxylase activity **(Samková, Dadáková and Pelikánová, 2013; Torracca et al., 2016)**.

Typically, these cheeses contain up to hundreds mg.kg$^{-1}$ of histamine, tyramine, putrescine, and cadaverine, up to tens mg.kg$^{-1}$ of 2-phenylethylamine as well as minor content of tryptamine **(Standarová et al., 2010)**. Cheeses also contain polyamines (Pas), such as agmatine, spermidine, and spermine **(Novella-Rodríguez et al., 2003)**.

The aim of this paper was to find out if the storage temperature and period could affect the BA content in smear ripened cheeses.

### Scientific hypothesis
The formation of BAs in cheeses is affected by the storage temperature – the higher the temperature, the higher the BA content.

The length of storage of cheeses affects their safety in terms of high BA content.

## MATERIAL AND METHODOLOGY
The cheese for testing was delivered as small rounds (diameter 45 mm, height 10 mm), where a 100 g package contained 5 pieces of these round portions. Prior to shipment, the cheeses ripened for 7 days, while the best before date (BBD) on the package was 28 days. A total of 5 batches of the product were analysed.

The samples were divided into three groups designated as (A), (B), and (C) and stored in different temperature regimes. Sampling and subsequent analyses were performed on the day of manufacture (A/0 = B/0 = C/0), at the end of BBD (A/35, B/35, C/35), two weeks after BBD (A/49, B/49), and eight weeks after BBD (C/91).

Samples in the temperature regime (A) were stored at 6 °C after production for the entire BBD (7 days before and 28 days after shipment), i.e. 35 days. These cheeses

were then stored for 14 days after the BBD expired at 6 °C, i.e. 49 days in total (6 °C/35 days → 6 °C /14 days). Samples in the temperature regime (B) were stored at 6 °C after production for 28 days (7 days before and 21 days after shipment), i.e. 28 days, then they were frozen and stored at -18 °C for 7 days. Subsequently, after BBD (35 days), the cheeses were stored at 6 °C for 14 days, i.e. 49 days in total (6 °C/28 days → -18 °C/7 days → 6 °C/14 days). Samples in the temperature regime (C) were stored at 6 °C after production for the entire BBD (7 days before and 28 days after shipment), i.e. 35 days. They were then frozen and stored for 49 days (7 weeks) at -18 °C. After 49 days of freezing, the cheeses were stored at 6 °C for 7 days, i.e. 91 days in total (6 °C/35 days → -18 °C/49 days → 6 °C/7 days).

The analyses were carried out in the laboratory of the Institute of Food Technology, Faculty of AgriSciences of Mendel University in Brno.

BA Assay: A cheese sample was grated to a particle size of about 3 mm and 10 g was weighed into an 85 mL plastic centrifuge tube. After addition of 20 mL of 0.1 M HCl and 0.5 mL solution of internal standard (1,7-diaminoheptane) with concentration of 1 mg.mL$^{-1}$, the sample was extracted with a disintegrant for 2 minutes. The suspension was centrifuged at 755 g for 10 minutes at 4 °C in order to separate the solid and fat. The supernatant was filtered and the solid was re-extracted by the same procedure. The combined extracts were added up to 50 mL with deionized water and filtered through a nylon membrane filter. The BA derivatization was carried out with dansyl chloride, where 1 mL of extract or standard was mixed with 0.5 mL of saturated $Na_2CO_3$ (pH adjusted to 11.2). In a 4 mL sample vial, 1 mL of derivatizing agent (5 mg of dansyl chloride in 1 mL of acetone) was added and stirred for 1 minute on an agitator. Derivatization was carried out for 1 hour at 40 °C without light access with occasional shaking at 15 minutes intervals. After derivatization, 250 µL of 10 mM ammonia was added to remove unreacted dansyl chloride and again stirred for 1 minute on the agitator. Ammonia reacted with excess dansyl chloride and the resulting reaction product was eluted before BA. Containers with dansyl chloride acetone solution and all standards and extracts were immediately after adding packaged in aluminium foil because of their photolability. After a 30-minute reaction, the hydrophobic amine derivatives were extracted with diethyl ether (3 x 1 mL) while the hydrophilic amino acid derivatives remained in the aqueous phase. The organic phase was evaporated to dry state with a stream of nitrogen and the evaporation residue was dissolved in 0.5 mL ACN or in 1 mL ACN (standard) and the solution was again filtered through a 0.45 µm nylon membrane filter and dosed on a chromatographic column. The amount of the injected real sample was modified as needed, in the case of standard it was 10 µL.

Biogenic amines were separated on a Zorbax Eclipse XDB C18 column (150 mm x 4.6 mm, particle size 5 µm) with Meta Guard ODS 2 pre-column (30 mm x 4.6 mm, particle size 5 µm) at a flow rate of 0.8 mL.min$^{-1}$ using the HP 1100 Chromatograph. Separation after derivatization with dansyl chloride was carried out by gradient elution with $H_2O$/ACN (time 0 – 23 minutes: $H_2O$ 35 – 0%, ACN 65 – 100%) followed by detection with a photometric UV/VIS detector at 254 nm. To identify the separated substances in the samples, a comparison of the retention times of the standards and the substances present in the sample was used. During the analysis, the UV spectra of the eluates were captured at peak and then compared with the spectra of the standard substances, and the identity of the substances was confirmed or refuted on the basis of the so-called identity factor.

All BA standards were supplied as hydrochlorides and their concentrations after derivatization with dansyl chloride were expressed in mg.kg$^{-1}$ of the original (fresh) sample (not per kg of dry weight) to better express the conditions at consumption. The BA concentration in a sample was corrected by the internal standard method. The internal standard was prepared by dissolving 100 mg of 1,7-diaminoheptane in 100 mL deionized water (concentration 1 mg.mL$^{-1}$).

The BA/PA standards stock solution was prepared as a mixed standard for all amines to be analyses (tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine) by dissolving 100 mg of each of the amines (in the form of hydrochloride) in 100 mL of deionized water (standard concentration of each amine 1 mg.mL$^{-1}$). The BA/PA standard working solution was obtained by mixing 0.5 mL BA/PA standard stock solution with 0.5 mL internal standard solution and subsequent treatment to a volume of 50 mL. The final concentration of each amine was 10 µg.mL$^{-1}$. The 0.1 M HCl prepared by dissolving 3.5 mL of 35% HCl in deionized water and filled to 1 litre was used as the extraction agent. The derivatizing agent was prepared by dissolving 5 mg of dansyl chloride in 1 mL of propan-2-one.

The following biogenic amines were determined: tyramine, histamine, 2-phenylethylamine, tryptamine, cadaverine, putrescine, spermidine, and spermine. The amine content of each sample was measured in duplicate.

### Statistical analysis

Statistical evaluation was performed in the Statistica Statsoft programme, version 12, and Microsoft Excel 2010. Basic statistical characteristics, such as mean and standard deviation of the mean were calculated.

In order to compare the BA content during the storage period within the given temperature regime, a simple scattering analysis method (ANOVA) including the Duncan post-hoc test was used. Normality was tested by the Shapiro-Wilk test. The proportion of factors (temperature and storage period including interactions) on the total variability of BA content in cheese was calculated using the general linear model (ANOVA with interactions).

## RESULTS AND DISCUSSION

At the beginning of storage (A/0, B/0, C/0) the total content of the monitored BA was 64.9 mg.kg$^{-1}$ of cheese (Figure 1). During ripening in storage in the temperature regimes (A) and (C), the total BA content first increased ($p$ <0.05) to 259.7 mg.kg$^{-1}$. Then it decreased ($p$ >0.05) to 190.4 mg.kg$^{-1}$ in regime (A) and to 140.7 mg.kg$^{-1}$ in regime (C).

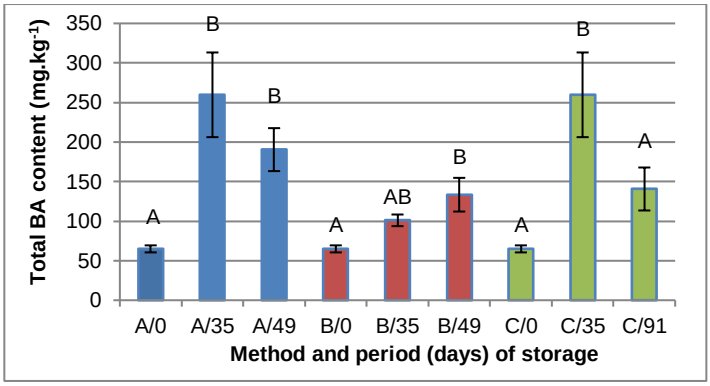


**Figure 1** Comparison of the total content of biogenic amines (mg.kg$^{-1}$) in smear ripened cheeses stored in different temperature regimes (A, B, C) and analysed on the production date (0), at the end of the best before date after 35 days of ripening (A/35: storage at 6 °C/35 days, B/35: Storage at 6 °C/28 days and at -18 °C/7 days, C/35: storage at 6 °C/35 days) and at the end of storage after 49 or 91 days of ripening (A: storage at 6 °C/35 days and at 6 °C/14 days, B: storage at 6 °C/28 days and at -18 °C/7 days and at 6 °C/14 days, C: storage at 6 °C/35 days and at -18 °C/49 days and at 6 °C/7 days). Averages marked with different letters are statistically different within a given factor (storage period) ($p$ <0.05); n = 15.

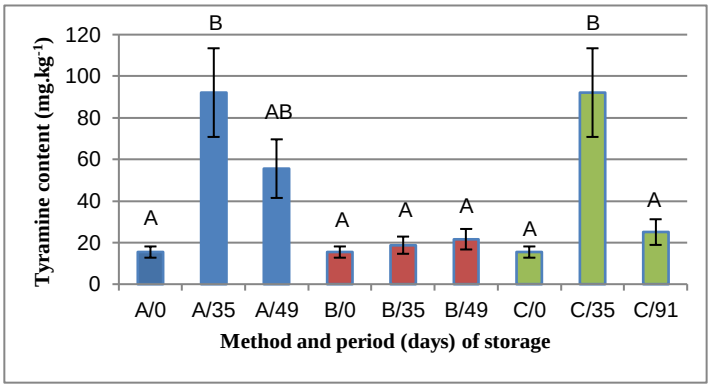


**Figure 2** Comparison of tyramine content (mg.kg$^{-1}$) in smear ripened cheeses stored in different temperature regimes (A, B, C) and analysed on production day (0), at the end of the best before date after 35 days of ripening (A/35: storage at 6 °C/35 days, B/35: storage at 6 °C/28 days and at -18 °C /7 days, C/35: storage at 6 °C/35 days) and at the end of storage after 49 or 91 days of ripening (A: storage at 6 °C/35 days and 6 °C/14 days, B: storage at 6 °C/28 days and at -18 °C/7 days and 6 °C/14 days, C: storage at 6 °C/35 days and at -18 °C/49 days and at 6° C/7 days). Averages marked with different letters are statistically different within a given factor (storage period) ($p$ <0.05); n = 15.

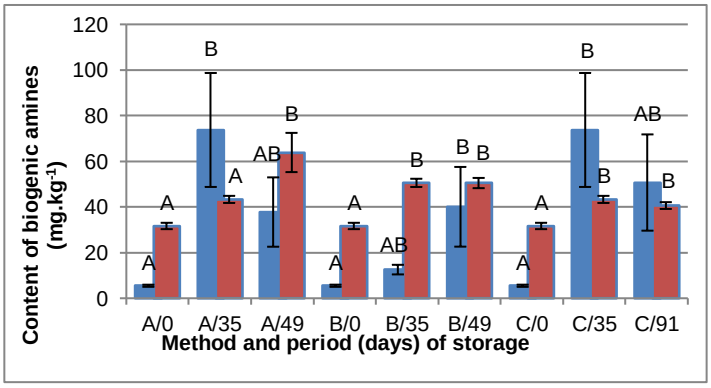


**Figure 3** Comparison of putrescine and polyamine content (mg.kg$^{-1}$) in smear ripened cheeses stored in different temperature regimes (A, B, C) and analysed on production day (0) at the end of the minimum shelf life after 35 days of ripening (A/35: storage at 6 °C/35 days, B/35: storage at 6 °C/28 days and at -18 °C/7 days, C/35: storage at 6 °C/35 days) and at the end of storage after 49 or 91 days of ripening (A: storage at 6 °C/35 days and 6 °C /14 days, B: storage at 6 °C/28 days and at -18 °C/7 days and 6 °C/14 days, C: storage at 6 °C/35 days and at -18° C/49 days and 6 °C/7 days). Averages marked with different letters are statistically different within a given factor (storage period) ($p$ <0.05); n = 15. Putrescine is marked blue, polyamines spermidine and spermine red.

The reduction in the total BA content of cheeses stored in temperature regime (C) was statistically significant ($p$ <0.05); lower total BA content was recorded at the end of storage (C/91) than at the end of BBD (C/35). When stored in regime (B), the total BA content was higher ($p$ <0.05) at the end of ripening (B/49) than on production date (B/0). The highest total BA value was recorded during storage in regime (A) after 35 days of ripening, namely 571.4 mg.kg$^{-1}$ of cheese.

Higher total BA content compared to ours was detected in smear ripened cheeses by **Pleva et al. (2014)**, namely up to 1,000 mg.kg$^{-1}$. In 5 samples they show even higher values (1,000 – 6,000 mg.kg$^{-1}$ of cheese). They ascribe the increased BA content to incorrect storage of cheese at stores. According to **Komprda et al. (2012)**, the impact of storage temperature on the variability of BA and PA content is 46% on average. The storage temperature has been reported by **Komprda et al. (2012)** for BA and PA variability has an average effect of 46%. Higher total BA content, as compared with our data, was also found by **Samková, Dadáková and Pelikánová (2013)** when cheeses were stored at 5 °C, namely 514 mg.kg$^{-1}$. After an additional two weeks of storage, the BA content further increased to 660 mg.kg$^{-1}$, with the highest recorded value of BA being 2,076 mg.kg$^{-1}$ of cheese. **Standarová et al. (2010)** for the same type of cheese, and at the same conditions at the end of BBD, detected 1,500 mg.kg$^{-1}$ BA; **Dičáková and Dudriková (2007)** 2,477 mg.kg$^{-1}$; **Rejchrtová (2015)** 593 mg.kg$^{-1}$ and 66 days after the production 1,218 mg.kg$^{-1}$, which are much higher values than our results. According to **Standarová et al. (2009)**, there are also differences in BA content depending on the month of cheese production.

During storage, in temperature regimes (A) and (C) the overall BA content decreased. The reduction in the overall BA content between the 6$^{th}$ and 7$^{th}$ week of storage was reported by **Standarová et al. (2010)** for cheeses stored at 5 °C. In contrast, no significant changes in the overall BA content of long-frozen (-18 °C) cheeses were found by **Andiç et al. (2010)**, and they explain this finding by slowing down or cessation of microorganism activity. In our experiment, there was apparently for these reasons a reduction ($p$ <0.05) in the overall content of the monitored BAs in regime (C).

Legislative limits on BA content in cheeses are currently not given. **Spanier, Bruin and Van Roode (1991)** state the content of histamine + tyramine + putrescine + cadaverine to be 900 mg.kg$^{-1}$ of cheese on maximum. This value was not exceeded in our experiment.

During ripening, 8 biogenic amines were monitored. As for tryptamine, it was not detected in cheese at the beginning of storage at all. Its content was found in cheese only after 35 days of ripening in regimes (A) and (C), averaging 2.7 mg.kg$^{-1}$. Under conditions of storage regime (B), tryptamine was not detected in cheeses at all. The same results were obtained by **Standarová, Borkovcová and Vorlová (2008)** when analysing 215 samples of cheeses consumed in the Czech Republic. Tryptamine content in these cheeses was low or tryptamine was not detected at all. Low, up to 5.9 mg.kg$^{-1}$, or undetectable amounts of tryptamine were also found by **Standarová et al. (2009),** however in the Niva cheese taken from the Czech distribution network. **Samková, Dadáková and Pelikánová (2013)** found at the end of BBD of the same type of cheese tryptamine at 5.3 mg.kg$^{-1}$, after two more weeks of storage, the content of 5 mg.kg$^{-1}$, which is more compared to our results. **Pleva et al. (2014)** just like us did not detect any tryptamine in smear ripened cheeses.

According to **Novella-Rodrigues et al. (2003)** and **Buňková et al. (2010)** cadaverine along with tyramine and putrescine belong among the most important BAs in ripened cheeses. As in our experiment, these authors recorded the highest cadaverine values at the end of BBD after 35 days of storage. However, the values we found (26.9 mg.kg$^{-1}$) were more than ten times lower than those of the authors above. Higher cadaverine contents in cheese compared to our results (up to 2,400 mg.kg$^{-1}$) were reported by **Pleva et al. (2014)** and **Standarová et al. (2010)** after 28 days of storage at 5 °C (>400 mg.kg$^{-1}$). Also, **Samková, Dadáková and Pelikánová (2013)** report, compared to us, a higher cadaverine content of 176 mg.kg$^{-1}$ at the end of BBD and 249 mg.kg$^{-1}$ two weeks after the end of BB with the same type of cheese.

As for histamine, the highest values in our experiment were detected at the end of BBD during storage at 6 °C, with an average content of 17.5 mg.kg$^{-1}$ of cheese and the highest recorded value of 49.5 mg.kg$^{-1}$. **Pleva et al. (2014)** report higher histamine content (373.8 mg.kg$^{-1}$) compared to ours for the same type of cheese. **Mayer, Fiechter and Fischer (2010), Standarová et al. (2010),** and **Loizzo et al. (2013)** also reported higher histamine content in smear ripened cheeses ranging between 168.3 mg.kg$^{-1}$ and 500 mg.kg$^{-1}$. **Rejchrtová (2015)** also detected higher histamine levels at the end of BBD compared to our results to be 110 mg.kg$^{-1}$ of cheese and after 66 days of manufacture to be 142 mg.kg$^{-1}$. **Samková, Dadáková and Pelikánová (2013)** found at the end of BBD when stored at 5°C the histamine content of 51.5 mg.kg$^{-1}$ of cheese and after two more weeks of storage 55.3 mg.kg$^{-1}$, which is also higher than our results. Decrease in the histamine content (similar to our experiment) between 30$^{th}$ and 60$^{th}$ day of ripening was reported by **Martuscelli et al. (2005)** in the Pecorino Abruzzese cheese. **Dalgaard et al. (2006)** report that short-term freezing had a significant effect on the subsequent decrease in histamine content (in muscle tissue of sea pike). This corresponds to our results when, in regime (B) at the end of BBD, the average histamine content was 8.0 mg.kg$^{-1}$ of cheese and in regime (A) 17.5 mg.kg$^{-1}$ of cheese. **Standarov, Borkovcová and Vorlová (2008)** report that the histamine content of 100 mg.kg$^{-1}$ of cheese may already induce intoxication in human organisms. However, this level of histamine content was not recorded in our experiment.

Cheeses typically contain up to tens of mg.kg$^{-1}$ of 2-phenylethylamine. In our experiment, at the end of BBD in cheeses stored at 6 °C (regime A), we have detected 5.4 mg.kg$^{-1}$ of 2-phenylethylamine and two weeks after BBD 1.4 mg.kg$^{-1}$. **Pleva et al. (2014)** reported higher content of 2-phenylethylamine in the same type of cheese than in our experiment, namely 11.6 mg.kg$^{-1}$. In contrast, **Samková, Dadáková and Pelikánová, (2013)** detected at the end of BBD in smear ripened cheeses a content of 2-phenylethylamine lower than ours**,** namely 4.3 mg.kg$^{-1}$. However, after two more weeks of storage compared to our data, the content of 2-phenylethylamine was higher (2.3 mg.kg$^{-1}$). **Standarová, Borkovcová and Vorlová**

**(2008)** reported that migraine can already be induced by 30 mg.kg$^{-1}$ of 2-phenylethylamine. This value was not recorded in our experiment.

## Tyramine

At the beginning of storage (A/0, B/0, C/0), the tyramine content in cheeses was 15.4 mg.kg$^{-1}$. During the ripening process (Figure 2), in storage in temperature regimes (A) and (C) there was first an increase ($p$ <0.05) of the tyramine content to 92.1 mg.kg$^{-1}$ of cheese, at the end of BBD after 35 days. At the end of storage in regime (A), lower values were recorded, namely 55.6 mg.kg$^{-1}$. In regime (C) the decrease ($p$ <0.05) of tyramine content was more pronounced, namely up to 25.1 mg.kg$^{-1}$ of cheese. During storage in regime (B), the tyramine content did not change in the course of ripening of the cheese ($p$ >0.05). The highest value of tyramine was recorded in our experiment after 35 days of ripening in regime (A), namely 291.7 mg.kg$^{-1}$ of cheese. The upper limit of tyramine content in food, according to **Halász et al. (1994)**, **Shalaby (1996)**, **Silla Santos (1996)** and **Eerola et al. (1997)** ranges from 100 to 800 mg.kg$^{-1}$ of food. According to **Latorre-Moratalla et al. (2008)**, people taking MAO inhibitors tolerate 50 – 100 mg tyramine, however problems can even be caused by 6 mg.kg$^{-1}$ **(Novella-Rodríguez et al., 2003)**. In samples stored at 6 °C (regime A), a "safe limit" of 100 mg.kg$^{-1}$ was exceeded in 5 out of 15 analysed samples at the end of BBD. Tyramine content >100 mg.kg$^{-1}$ was not detected during storage in temperature regime (B) for any cheese.

In the same type of cheese, **Standarová et al. (2010)** detected the highest tyramine concentration between the 5$^{th}$ and 6$^{th}$ week of storage at 5 °C, which corresponds to our results (A/35). **Rejchrtová (2015)** reported at the end of BBD in the same type of cheese at 5 °C higher tyramine content compared to ours (>800 mg.kg$^{-1}$). **Samková, Dadáková and Pelikánová (2013)** likewise recorded a slightly higher average tyramine values than in our experiment at 5 °C at the end of BBD, namely 140 mg.kg$^{-1}$. After two more weeks of storage the value was 163 mg.kg$^{-1}$, while the highest tyramine reading after extended storage in their experiment was 469 mg.kg$^{-1}$ of cheese.

In our experiment, the tyramine content as well as histamine and total BA content decreased between the 5$^{th}$ and 7$^{th}$ week of storage (A/49). According to **Leuschner et al. (1998)**, one possible explanation can be the fact that some microorganisms have the ability to degrade tyramine and histamine. In testing 32 strains of *B. linens* and coryneform bacteria, it was found that 21 of them showed histamine or tyramine oxidase activity. These authors further state that *Brevibacterium linens* (LTH 456 and LTH 3686) were able to reduce the tyramine and histamine content for Munster smear ripened cheese by 55 to 70%. According to **Bergey and Holt (1994)**, **Leuschner, Heidel and Hammes (1998)** and **Dasu et al. (2006)**, also *Pseudomonas* sp., *Seratia marcescens*, *Kocuria varians*, and other bacteria can decompose the created BA. **Komprda et al. (2007)** found an increasing content of tyramine with time in a Dutch type cheese from two different manufacturers. **Standarová et al. (2009)** reported similar results for cheese with blue mould in dough and **Martuscelli et al. (2005)** for Pecorino Abruzzese cheese. The latter authors observed the most significant increase in tyramine content between the 14$^{th}$ and 30$^{th}$ day of ripening, which corresponds to our results.

## Putrescine

At the beginning of storage (A/0, B/0, C/0), the putrescine content in the cheeses was 5.5 mg.kg$^{-1}$. During ripening (Figure 3), when stored in temperature regime (A) and (C), the content of putrescine in cheeses first increased to 73.7 mg.kg$^{-1}$ ($p$ <0.05). After expiration of BBD and further storage, putrescine content values in cheeses were lower, namely 37.8 mg.kg$^{-1}$ in regime (A) and 50.7 mg.kg$^{-1}$ in regime (C). In temperature regime (B), putrescine content increased ($p$ <0.05) during storage, when after 49 days of storage (B/49) a higher putrescine content than on production date (B/0) was recorded.

Higher content of putrescine, compared to our data, was found by **Pleva et al. (2014),** when putrescine in smear ripened cheeses was detected at >2,000 mg.kg$^{-1}$. **Standarová et al., (2010)** also reported that the putrescine content was higher, when after 28 days of ripening at 5 °C its content was 212 mg.kg$^{-1}$ in the same type of cheese. In our experiment, too, the highest average putrescine content was found after 35 days of ripening at 6 °C. However, the average content was lower at 73.7 mg.kg$^{-1}$, while the highest recorded value was 320 mg.kg$^{-1}$ of cheese. **Samková, Dadáková and Pelikánová (2013)** detected a higher average putrescine content of 104 mg.kg$^{-1}$ in smear ripened cheeses at the end of BBD when stored at 5 °C. After two more weeks of storage, the putrescine content increased even more to 137 mg.kg$^{-1}$.

In our experiment, however, the putrescin content decreased ($p$ >0.05) after a further 2 weeks of storage at 6 °C (A/49). In contrast, in regime (B) the putrescine content of cheeses increased, when at the end of storage (B/49) a higher ($p$ <0.05) content of putrescine than at the production date (B/0) was detected. An increase in the putrescine content during storage (of tuna) at low temperatures (-18 °C) was also reported by **Ben-Gigirey et al. (1998)**. However, during long-term freezing (regime C), there was a decrease in the putrescine content in cheeses, which is probably related to the decrease of enzymatic activity of microorganisms **(Andiç et al., 2010)**.

## Polyamines

As regards PAs (spermidine and spermine), their content in cheeses was 31.7 mg.kg$^{-1}$ at the beginning of storage (A/0, B/0, C/0). During storage, the PA ($p$ <0.05) content has increased in all temperature regimes. At the end of the storage period, the cheeses had a higher ($p$ <0.05) PA content than on the day of production (Figure 3).

At present, PAs and their precursor putrescine are classified differently from the BA group. PAs may originate in a different metabolic pathway and are characterized by different biological functions. **Pleva et al. (2014)** show higher PA values compared to ours (A/35) for the same type of cheese, namely 55.8 mg.kg$^{-1}$ on average. In contrast, **Samková, Dadáková and Pelikánová (2013)** measured lower values of PAs at the end of BBD compared to ours, namely 33 mg.kg$^{-1}$ for cheeses stored at 5 °C. After two more weeks of storage, they found a PA

content of 48.2 mg.kg$^{-1}$ of cheese, which is also less compared to our values (A/49).

## CONCLUSION

Our hypothesis regarding the effect of temperature on BA formation in cheeses was confirmed. Their content was higher at 6 °C than when stored at freezing temperatures. Freezing does not completely deactivate the enzymes, as enzyme reactions are slowly taking place even at freezing temperatures. However, it is important to keep in mind that low temperatures recommended for food storage were used in our experiment.

The highest ($p$ <0.05) total BA content was found when storing the cheeses at the end of BBD (best before date) after 35 days (259.7 mg.kg$^{-1}$) in storage regime (A) or (C). During storage in regime (B), the total BA content ($p$ <0.05) after 49 days of storage was higher than on the production date (B/0). During storage, the tyramine content in regime (B) did not change ($p$ >0.05), while in the temperature regimes (A) and (C), the highest levels of tyramine and putrescine content were recorded in cheeses at the end of BBD after 35 days ripening (92.1 mg.kg$^{-1}$, resp. 73.7 mg.kg$^{-1}$). The content of polyamines in cheeses was higher ($p$ <0.05) at the end of storage: 63.9 mg.kg$^{-1}$ in storage regime (A), resp. 50.5 mg.kg$^{-1}$ in storage regime (B) and 40.6 mg.kg$^{-1}$ in storage regime (C), than at the beginning (31.7 mg.kg$^{-1}$).

Analyses performed (ANOVA with interactions) show that greater effect on the content of BAs (tyramine, putrescine) and PAs had the period of storage rather than the method of storage (temperature regime).

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