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# BIOGENIC AMINE CONTENT IN MOULD CHEESE DURING STORAGE

## ZAWARTOŚĆ AMIN BIOGENNYCH W SERZE PLEŚNIOWYM W TRAKCIE PRZECHOWYWANIA

**Abstract:** The aim of this research was to study the formation of seven biogenic amines (histamine, agmatine, spermine, spermidine, cadaverine, putrescine and tyramine) in three commercial mould cheeses from three different producers from the area of the Central Europe during 8-week storage in refrigerator at 6 ± 2 °C. The analysis of biogenic amines was made every week during 8-week of storage. Biogenic amines were extracted from the mould cheese by diluted HCl and determined using ion-exchange chromatography with post-column ninhydrin detection. Spermidine, spermine, putrescine and cadaverine were detected in tested mould cheeses. Spermidine was quantitatively the most important biogenic amine in all samples. While spermidine was detected immediately after purchase of samples, the rest of detected biogenic amines were developed during storage. The amount of putrescine was mostly increased during storage while the concentration of spermidine was decreased during storage. However, after 8 weeks of storage all samples contained toxicologically insignificant concentrations of detected biogenic amines in comparison with EU legislation and scientific literature and can be considered to be safe for human health.

**Keywords:** biogenic amine, mould cheese, ion-exchange chromatography

Biogenic amines are non-volatile low molecular nitrogen organic bases possessing biological activity. These compounds can have aliphatic, aromatic and heterocyclic structure. Biogenic amines, mainly polyamines, are indispensable parts of cells being essential for the regulation of nucleic acid function, stabilization of membranes, cell growth and proliferation, blood pressure regulation, etc. Some biogenic amines serve as free radical scavengers and antioxidants. Biogenic amines contained in food originate especially from the decarboxylation of the corresponding amino acids by micro-organisms. Decarboxylation activity is typical especially for families *Lactobacillaceae* and *Enterobacteriaceae* [1–3]. Consumption of food with high biogenic amine content (especially fish, meat and cheese products, wine, beer and other fermented food) may

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result in various health problems. They can cause eg nausea, vomiting, diarrhoea, palpitation, headache, depression, dizziness, hypo- or hypertension and, in extreme cases, anaphylactic shock, heart attack, etc. [1, 4–7]. Furthermore, some biogenic amines can participate in the formation of carcinogenic nitrosamines [5].

Camembert-type cheese is a dairy product which belongs to the semi-soft/soft mould ripened cheese. It is covered on the surface by mould *Penicillium camemberti*. The manufacture includes following main steps: milk standardization, pasteurization, starter culture and mould inoculation, renneting, whey removal, forming, salting, wrapping and ripening. The ripening period last about 2–3 weeks [8]. The ripening of cheese is characterized mainly by hydrolysis of caseins which leads to the increase of free amino acid content. Some of these amino acids can be subjected to decarboxylation to form biogenic amines. BA production in cheese can be influenced by many factors, eg by kind of starter culture strain, bacterial activity, pH of cheese, salt and fermentable saccharide concentration, storage temperature, ripening time, etc. [9, 10]. The content of BA in mould cheese could be affected also by deamination of amino acids by the enzyme activity of present moulds.

In the Czech Republic, only the law for histamine content in fish adopted from EU is valid. According to it, histamine content in 7 of 9 fish samples must not exceed 100 mg · $kg^{-1}$; at the same time, 2 samples of 9 can contain more than 100 mg · $kg^{-1}$ histamine, but less then 200 mg · $kg^{-1}$ [11]. Nevertheless, there are nowadays no other thresholds for the other biogenic amines. Previously, there were also limits for tyramine in various foods such as cheese (200 mg · $kg^{-1}$) or red wine (50 mg · $kg^{-1}$) [12].

There is numerous information about biogenic amines in cheese available in the literature [7, 13–15]. However, there was not found any paper dealing neither with the mould cheese nor with moulds producing biogenic amines. Hence, the aim of this study is to investigate the formation and the amount of biogenic amines in soft mould ripened cheeses obtained from trade network during 8-week storage in refrigerator at 6 ± 2 °C.

## Materials and methods

### Samples

Three different commercial brands of mould cheese (Sedlcansky hermelinek, Karel IV Camembert, Stribrnak) were assessed by biogenic amine content. All these samples were soft mould ripened cheeses covered by white mould (*Penicillium camemberti*). Sedlcansky hermelinek (H) was manufactured by Povltavske mlekarny, a.s. (Sedlcany, Czech Republic), Karel IV Camembert (C) was produced by Polser Sp. z.o.o. (Siemiatycze, Poland) and mould cheese Stribrnak (S) was made in MV Oberfranken – West eG (Wiesenfeld, Germany). All samples were stored in the refrigerator at 6 ± 2 °C until the analysis.

### Chemical analysis of samples

Chemical analysis of samples was made immediately after purchase (0 day of storage) and then after 28 and 56 days of storage. Samples were characterized by dry

matter and fat content, by pH and crude protein (see Table 1). Dry matter content was determined by drying at 102 ± 2 °C to a constant weight according to ISO 5534:2004 [16]. The pH value was measured by inserting of glass electrode THETA 90 HC 113 (Gryf, Havlickuv Brod, Czech Republic) of pH-meter Gryf 208 L (Gryf, Havlickuv Brod, Czech Republic) into the water solution of sample (10 g of sample disintegrated in 30 $cm^{-3}$ of distilled water) at 20 ± 1 °C. Fat content was determined by the acidobutyric method of van Gulik. Crude protein was assayed by analysing total nitrogen by Kjeldahl method using distillation unit Pro-Nitro A (JP SELECTA, Barcelona, Spain) and calculating crude protein content as total nitrogen multiplied by 6.38.

## Biogenic amine extraction

Ten grams of sample was extracted with 25 $cm^{-3}$ of 0.1 mol · $dm^{-3}$ HCl in stomacher for 7 minutes. After extraction, the homogenized sample with extraction solution was centrifugated in 50 $cm^3$ centrifugal tubes in centrifuge Z 300 K (HERMLE Labor-technik GmbH, Wehingen, Germany) at 6500 rpm at 4 °C for 30 minutes. The supernatant was filtered and evaporated by using rotary vacuum evaporator RVO 400 A (Ingos, Prague, Czech Republic) to the syrup consistency. The rest after evaporation was dissolved in sodium-citrate buffer (pH = 2.2) in 10 $cm^3$ volumetric flask. The solution was filtered through the nylon membrane filter (0.45 μm) and loaded into analyzer.

## Biogenic amine analysis

Isolated biogenic amines were analyzed by using ion-exchange chromatography (column 55 × 3.7 mm filled with ion exchanger OSTION Lg ANB) equipped with post-column ninhydrin derivatization and spectrophotometric detection (λ = 570 nm). The analysis was made by using Amino Acid Analyser AAA400 (Ingos, Prague, Czech Republic). The buffer system, protocols of the analysis (elution programs) and the process of ninhydrin reagent preparation were used as described in Standara et al [6]. A mixed standard solution of 7 biogenic amines (histamine, agmatine, spermine, spermidine, cadaverine, putrescine, tyramine) in sodium-citrate buffer (pH = 2.2) with the concentration of 500 nmol · $cm^{-3}$ of each amine was prepared. Biogenic amine standards were obtained from Sigma-Aldrich (St. Louis, USA). All reagents for AAA and the ion exchanger for the column were purchased from Ingos (Prague, Czech Republic). Two samples of each brand of mould cheese were investigated every week during 8-week storage and the whole extraction procedure and biogenic amine analysis were always made at least twice for each sample.

## Statistical analysis

Results obtained by chemical analysis were statistically evaluated using parametric t-test. Results were significantly different when $p < 0.05$. The dependency of BA

amount on the storage time was also evaluated using regression analysis (least squares method). Coefficient of correlation (r) for chosen model expressing changes in BA concentration depending on storage time was calculated.

## Results and discussion

The formation of seven biogenic amines (histamine, agmatine, spermine, spermidine, cadaverine, putrescine, tyramine) in three commercial mould cheeses during 8-week storage in refrigerator at 6 ± 2 °C was investigated. Results obtained by basic chemical analysis of mould cheeses are presented in Table 1. The analyses showed that there were not significant differences ($p \geq 0.05$) in dry matter content during 8-week storage in refrigerator at 6 ± 2 °C while values of pH increased ($p < 0.05$) during ripening of mould cheeses. Growing pH values during storage can be explained by protein hydrolysis and ammonia creation together with the utilization of lactic acid by present microorganisms.

Table 1

Chemical analysis of mould cheeses after 0, 28 and 56 days of storage in refrigerator at 6 ± 2 °C

| Sample* | Storage time [days] | DM** [% w/w] | Fat [% w/w] | pH [-] | CP* [% w/w] |
|---|---|---|---|---|---|
| H | 0 | 46.88 ± 1.08 [a] | 23.8 ± 0.4 | 6.67 ± 0.08 [a] | 17.98 ± 0.43 |
| | 28 | 46.75 ± 0.32 [a] | | 7.85 ± 0.05 [b] | |
| | 56 | 47.12 ± 0.94 [a] | | 8.03 ± 0.04 [c] | |
| C | 0 | 54.29 ± 0.79 [a] | 33.3 ± 1.8 | 7.06 ± 0.10 [a] | 18.16 ± 0.17 |
| | 28 | 54.71 ± 0.59 [a] | | 7.64 ± 0.01 [b] | |
| | 56 | 54.95 ± 1.51 [a] | | 7.57 ± 0.02 [c] | |
| S | 0 | 40.29 ± 1.38 [a] | 13.8 ± 0.4 | 6.76 ± 0.04 [a] | 22.70 ± 0.21 |
| | 28 | 40.98 ± 0.34 [a] | | 7.74 ± 0.05 [b] | |
| | 56 | 40.80 ± 0.82 [a] | | 7.79 ± 0.05 [b] | |

* H – Sedlcansky hermelinek, C – Karel IV Camembert, S – Stribrnak, DM – Dry matter content, CP – crude protein content.

** Means in a box followed by at least one similar superscript letter are not significantly different ($p \geq 0.05$).

In this study, three different commercial brands of mould cheese were investigated in terms of their biogenic amine content. Immediately after purchase, all mould cheeses contained only spermidine but only in negligible amounts. Samples S included also small amounts of spermine and putrescine and mould cheese S contained putrescine. During storage the contents of tested biogenic amine changed. In the majority of cases, the amounts of detected biogenic amines fluctuated slightly during first 5 weeks of storage. Then, there were observed different trends in concentrations of various biogenic amines. While the amount of spermidine was mostly slightly decreased, the concentrations of putrescine substantially increased. The decline in spermidine concentration in cheese during ripening, was observed by Novella-Rodriguez et al [17]. The

rise of putrescine amount was obvious especially after 5-week storage (samples H, S), respectively after 7-week storage (sample C). This increase is evident from the Fig. 1 where the dependence of putrescine amount on the storage time is shown. Exponential model describes this dependence more accurately and at a better fitting than the other models did. There were also detected cadaverine after 5-week storage in sample S and its concentration slightly fluctuated during the further storage. In sample C cadaverine was created after 7 weeks of storage. Agmatine, tyramine and histamine were not found in any of the tested samples. These conclusions are in agreement with some authors who also did not detect histamine in cheese. Karovicova et al [18] did not determine histamine in Cottage cheese or spreadable processed cheeses.

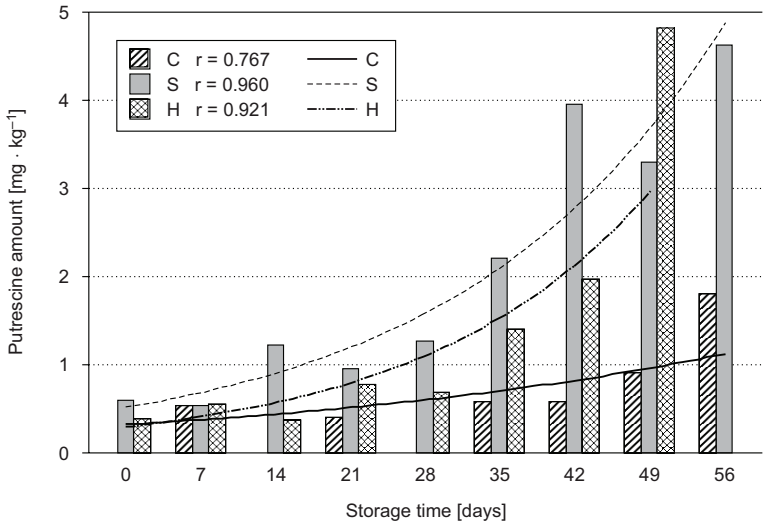


Fig. 1. The dependence of putrescine amount on storage time in 3 tested mould cheeses: H – Sedlcansky hermelinek, C – Karel IV Camembert, S – Stribrnak; r – coefficient of correlation for chosen model expressing changes in BA concentration depending on storage time

Total biogenic amine content is presented in Table 2. In samples C and S it increased mostly during storage period, while in sample H slightly fluctuated. At the end of the monitored period, concentrations of detected biogenic amines were negligible in comparison with toxic levels presented in available literature.

Results obtained in this study were significantly lower (the highest amine concentration was about 8 mg · $kg^{-1}$) when compared with some observations of other authors. Roig-Sagues et al [10] or Pinho et al [9], respectively, detected BA in 20 various cheese varieties in Spain or in ovine cheese Azeitao, respectively, concentrations about tens or hundreds of mg · $kg^{-1}$. On the other hand, Novella-Rodriguez et al [17] found similar low amounts of BA in some kinds of cheese in Spain as in this study. This wide variability of BA concentration of different cheese varieties may result from the type of cheese, the ripening time, the conditions of the

manufacturing process, the present microflora (starter culture but mainly non-starter lactic acid bacteria), etc. [17, 19].

Table 2

Total biogenic amine content of three tested mould cheeses during ripening

| Storage time [days] | Total BA concentration of cheese [mg · kg$^{-1}$] | | |
|---|---|---|---|
| | Sample H | Sample C | Sample S |
| 0 | 6.0 | 6.4 | 7.8 |
| 7 | 8.2 | 8.2 | 8.2 |
| 14 | 7.4 | 5.9 | 9.0 |
| 21 | 6.4 | 7.8 | 6.0 |
| 28 | 7.5 | 7.4 | 7.3 |
| 35 | 6.8 | 10.4 | 9.8 |
| 42 | 5.0 | 8.7 | 10.0 |
| 49 | 5.4 | 8.4 | 9.9 |
| 56 | 7.6 | 13.0 | 10.0 |

* H – Sedlcansky hermelinek, C – Karel IV Camembert, S – Stribrnak.

Insignificant concentrations of these nitrogenous compounds in this research would be explained by the presence of microorganisms with low decarboxylation activity and by deamination of amino acids by present enzymes of moulds. Also, the conditions for their growth or BA production could be not suitable. Values of pH of all samples were always relatively high (see Table 1) and according to Halasz et al [23] the optimum pH for decarboxylase formation, and, therefore, for BA production is in more acidic environment. Higher values of pH together with low storage temperature were sufficient for keeping samples of good quality in terms of biogenic amine content. Additionally, good hygienic condition in cheese production should be maintained to avoid the outbreak of food poisoning.

## Conclusions

The formation of biogenic amines in mould cheese during 8-week storage at 6 ± 2 $^{o}$C was studied. Spermidine, spermine and putrescine were detected in samples immediately after purchase, while cadaverine was created during storage of samples. Concentrations of spermine, spermidine and putrescine slightly fluctuated till five week of storage and, then, the amount of putrescine substantially increased while the concentration of spermidine decreased. Histamine, agmatine and tyramine were not detected in any of the samples. Before and also after the expiration date all detected biogenic amines were present in only negligible concentrations. It can be concluded that these low amounts of biogenic amines are not toxic for healthy people and, therefore, these mould cheeses are not danger as regards biogenic amine poisoning.

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## ZAWARTOŚĆ AMIN BIOGENNYCH W SERZE PLEŚNIOWYM W TRAKCIE PRZECHOWYWANIA


**Abstrakt:** Celem pracy było zbadanie syntezy siedmiu amin biogennych (histaminy, agmatyny, sperminy, spermidyny, kadaweryny, putrescyny i tyraminy) w trzech komercyjnie dostępnych serach pleśniowych pochodzących od różnych producentów z Europy Środkowej w czasie 8-tygodniowego przechowywania w lodówce w temperaturze 6 ± 2°C. Oznaczenia poziomu amin biogennych wykonywano raz w tygodniu. Aminy biogenne były izolowane z sera pleśniowego przez rozcieńczony HCl i oznaczane metodą chromatografii jonowymiennej i postkolumnowej reakcji ninhydrynowej. W badanych serach wykryto obecność spermidyny, sperminy, putrescyny i kadaweryny. W największych ilościach występowała spermidyna. Związek ten wykrywano w świeżo wyprodukowanym serze, natomiast pozostałe aminy pojawiały się stopniowo w czasie przechowywania. Największy wzrost stężenia w czasie przechowywania stwierdzono w przypadku putrescyny. Natomiast poziom spermidyny zmniejszał się w czasie przechowywania. Po 8 tygodniach przechowywania badane sery zawierały jednak nieznaczne ilości amin biogennych, w stężeniach dopuszczalnych przez normy UE i bezpiecznych dla ludzkiego zdrowia.



**Słowa kluczowe:** aminy biogenne, ser pleśniowy, chromatografia jonowymienna