Effect of Some Factors on the Biogenic Amines and Polyamines Content in Blue-Veined Cheese Niva

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Abstract: We evaluated the effect of some factors (batch, producer, storage and amine distribution in cheese) on the biogenic amines and polyamines contents and microbial counts (*Enterobacteriaceae*, enterococci, lactic acid bacteria) in blue-veined cheese Niva. The tyramine content was increasing ($P < 0.01$) with storage time; at storage day 29, it reached 298 mg/kg and exceeded the toxicological limit. Cadaverine and the sum of biogenic amines also increased significantly ($P < 0.01$) and the latter reached up to 900 mg/kg, i.e. the established toxicological limit. The contents of the quantitatively most important biogenic amines (tyramine and cadaverine) and sum of biogenic amines varied according to batch and producer in the ranges of 3.0–337 mg/kg, 3.0–705 mg/kg, and 33–920 mg/kg, respectively. Considerably higher ($P < 0.01$) contents of tyramine, cadaverine and sum of biogenic amines and higher counts of enterococci and *Enterobacteriaceae* were found in the edge samples in comparison with the cheese core. The microorganisms were identified as *E. faecalis* and *E. faecium*.

Keywords: biogenic amines; polyamines; blue-veined cheese; HPLC; PCR

INTRODUCTION

Biogenic amines (BAs; histamine, tyramine, tryptamine, cadaverine) in foods are mainly produced by the microbial decarboxylation of amino acids (Silla-Santos 1996). Polyamines (PAs; putrescine, spermidine and spermine) are contemporarily considered as a distinct group as they can also be produced by another metabolic pathway and have specific physiological action. BAs have been implicated in food poisoning, usually associated with eating fermented foods that contain large amounts of these substances. Cheese provides an ideal environment for the production of BAs but the amine concentration differs widely and depends on several factors such as time of ripening, storage temperature, starter culture, part of cheese and microflora (Komprda *et al*. 2008).

The aim of this study was to evaluate some factors influencing the BA and PA content in blue-veined cheese Niva such as producer, batch, time of storage and BA distribution in cheese and correlation between the concentration of BAs and the presence of selected bacteria.

MATERIAL AND METHODS

Cheeses. Samples under analysis ($n = 66$) were taken from products available for retail sale. Cheeses from four different producers were sampled within two days after delivery to the market.

Methods. BAs and PAs were extracted from the food matrix using hydrochloric acid. Subsequently, they were analysed as dansyl derivatives by quantitative RP-HPLC with gradient elution and fluorescence detection (Paulsen *et al*. 1997). Chemical composition of cheese was determined from the homogenised sample: NaCl content was investigated according to Czech standard ČSN ISO 5943 and pH according to ČSN 57 0107. Water activity (a_w) was measured by an AW TH 500 instrument (Novasina, Switzerland). Cheese samples of cheese for microbial analyses were prepared ac-

Table 1. Contents of biogenic amines and polyamines of samples from Czech market (n = 26)

Amines	Producer				Total (mg/kg)	
	A	B	C	D	means contents	range
Histamine	22.0	20.3	11.0	16.0	17.3	2.0–28.4
Tyramine	170	23.5	33.9	84.2	77.9	2.9–337
Cadaverine	43.1	413	28.1	268	188	3.0–705
Tryptamine	3.0	6.0	1.0	3.8	3.5	ND–5.9
Σ of BA[a]	238	463	74.0	372	287	33.1–920
Putrescine	18.9	30.3	17.9	31.0	24.5	2.0–61.1
Spermidine	5.0	4.3	1.7	3.0	3.5	ND–7.8
Spermine	2.2	2.3	1.7	2.7	2.2	ND–7.0
Σ of PA[b]	26.1	29.8	21.3	36.7	30.2	5.5–65.9

ND – non detected; [a]sum of biogenic amines (histamine + tyramine + cadaverine + tryptamine); [b]sum of polyamines (putrescine + spermidine + spermine)

cording to ČSN ISO 6887-1. They were tested for enterococci on the Slanetz-Bartley Agar (Oxoid) at 37°C for 48 h, *Enterobacteriaceae* according to ČSN ISO 21528-2, lactic acid bacteria (LAB) according to ČSN ISO 15214. A PCR method was used for genotypic confirmation of enterococci (Cupáková *et al*. 2005). Subsequently, *Enterococcus faecalis* and *Enterococcus faecium* were identified by the PCR method of Jackson *et al.* (2004).

Statistical evaluation. Means of the two measurements were used in statistical evaluation. Statistical software STAT Plus, VRI Brno, Czech Republic, was used.

RESULTS AND DISCUSSION

The contents of individual BAs and PAs in cheese Niva from the retail store supplied by four different producers are presented in Table 1.

Cadaverine (3.0–705 mg/kg, the mean 188 mg/kg) and tyramine (3.0–337 mg/kg, the mean 78 mg/kg) were the most abundant amines. Novella-Rodríguez *et al.* (2003) found in Spanish blue cheeses more than 2100 mg/kg of cadaverine and 1585 mg/kg of tyramine. Despite the fact that the toxicological limits for individual BAs are difficult to establish, the value of 100 mg/kg of food suggested for tyramine (Silla-Santos 1996) is the most commonly used upper limit. Only 3 of 28 samples can be considered from this viewpoint. The sum of measured amines (BAs+PAs) ranged from 38 mg/kg to 986 mg/kg in the present experiment. As reported by Spanier *et al. (*1991), the level for 900 mg/kg of histamine + tyramine + cadaverine + putrescine is considered as a safety limit. Only one of our samples reached this value.

The amines content and profile in Niva cheese varied with producer (Table 1). The contents of individual amines and the BA sum in the present experiment were significantly ($P < 0.01$) lower in the products of producer C, while the contents of tyramine and cadaverine were significantly higher ($P < 0.01$) in cheese supplied by producers A and B, respectively.

The batch factor influenced considerably the BA and PA content of the cheeses and this effect was associated with month in which cheese had been produced (Figure 1). Differences were found in the contents of cadaverine, tyramine and the sum of BAs between cheeses produced by the same pro-

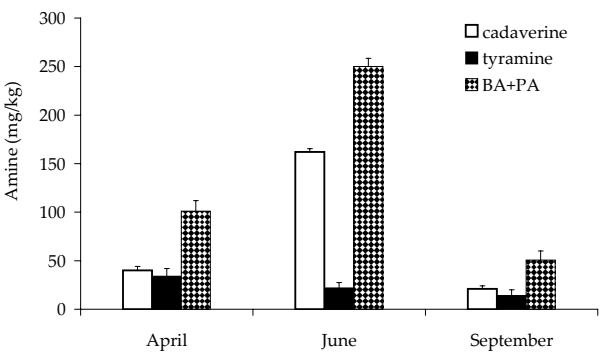

Figure 1. Effect of batch on the content of amines

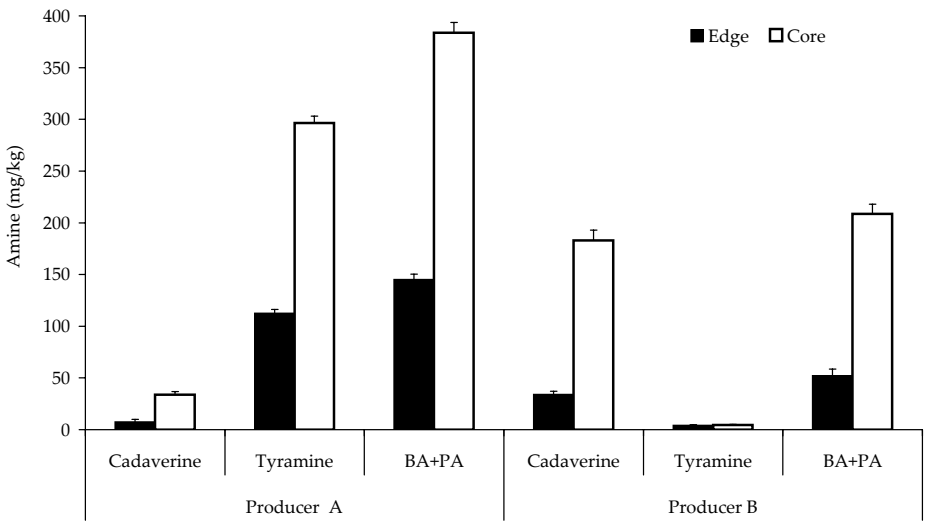

Figure 2. Comparison of amine content in the core and edge samples of two batches of different producers

ducer (**B**) in April, June and September ($P < 0.01$). As secondary contamination of milk and cheese differed between months, contamination by the decarboxylating bacteria cannot be omitted.

The contents of tyramine, cadaverine and BA sum in cheese samples from different producers (A, B) are shown in Figure 2. The BA contents in the edge and core samples are compared. A higher BA content ($P < 0.01$) reflects a higher tyramine (A) ($P < 0.01$) or cadaverine content (B) ($P < 0.01$). Consistent results have been reported by Komprda *et al*. (2008) in Edam cheese. The NaCl concentration, pH and a_w values did not markedly differ between the cheese core and edge samples in this experiment. Higher ($P < 0.01$) counts (in log CFU/g) of enterococci and *Enterobacteriaceae* were found in the edge part compared with the cheese core (5.87 vs. 3.95 and 6.57 vs. 3.95, respectively). The isolates were identified by the PCR assay as *E. faecalis* and *E. faecium.* As enterocci belong to the confirmed producers of tyramine and *Enterobacteriaceae* are known to produce cadaverine, these findings may contribute to a better understanding of BA distribution in blue-veined cheese. LAB contents were non-significantly ($P > 0.05$) higher in the cheese core.

In the present experiment, we simulate storage at market display conditions. Samples from two producers (A, B) were stored at 8°C (as recommended by the producer) in accordance with the date of durability (29 days). The content of all BAs tended to increase with increasing time of storage. The tyramine content was rising ($P < 0.01$) with increasing time of storage, reaching 298 mg/kg (producer A) on day 29. Cadaverine and the sum of BA also showed a significant upward trend and the sum BA (producer B) reached a toxicologically interesting value of 900 mg/kg.

CONCLUSION

Higher counts of enterococci and *Enterobacteriaceae* in the edge part of Niva in comparison with the cheese core may be the reason for higher contents of the quantitatively most important BAs, tyramine and cadaverine. The effect of batch, producer and the time of storage on the BAs content was demonstrated. The tyramine content and sum of BAs may exceed the established toxicological limit. Food intolerance and/or allergy sufferers and patients on monoamine oxidase inhibitors should avoid the consumption of blue-veined cheese.

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