Contents of Some Biologically Active Amines in a Czech Blue-vein Cheese

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Abstract

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Biogenic amines (histamine, tyramine, tryptamine, phenylethylamine, cadaverine) including biologically active polyamines (putrescine, spermidine and spermine) were determined by HPLC method after 7, 21, 35, and 49 days of ripening in the core (C) and edge (E) samples of a blue-veined cheese, popular in the Czech Republic under the trade mark Niva, produced in the three consecutive months (October, November, December) from pasteurised cow milk using *Penicillium roqueforti* spores; two vats were produced in each month. The cheese vat, including the production period, accounted for ($P < 0.05$) one third and two thirds of the explained variability of the sum of biogenic amines and the sum of polyamines, respectively. The ripening time was significant ($P < 0.05$) from this viewpoint only in the case of the sum of biogenic amines (nearly half of the explained variability). Putrescine and spermidine contents in cheese did not change ($P > 0.05$), spermine content even decreased ($P < 0.05$) with increasing time of ripening. Tyramine content (Y, mg/kg) in the core samples increased linearly with increasing time of ripening (X, days), $Y = 6.3 + 11.69X$, $R^2 = 0.26$, $P < 0.001$, contrary to the edge part where tyramine content did not change ($P > 0.05$). At the end of ripening (49 days), tyramine was quantitatively the most abundant amine (the mean and median 380 mg/kg and 289 mg/kg, respectively), its content in different cheeses (vats) varying from 10 mg/kg to 875 mg/kg. Cadaverine concentration varied between 3 mg/kg and 491 mg/kg (the mean 114, median 56 mg/kg). The levels of other biogenic amines and polyamines (with the exception of putrescine in the edge part of one of the December vats: 117 mg/kg) were very low even at the end of ripening. Tyramine contents at the end of ripening in the core-samples were higher ($P < 0.01$) in comparison with those in the edge-samples, contrary to histamine, cadaverine, putrescine, and spermine contents.

Keywords: tyramine; cadaverine; polyamines; blue-vein cheese; *Penicillium roqueforti*

Niva is a typical Czech variant of the internal-mould cheeses, produced under the use of specific strains of the *Penicillium roqueforti*. As a high-protein-containing ripening foodstuff, it belongs to the products where the degradation of proteins during ripening leads to the accumulation of free amino acids, which can be converted (due to the activity of bacterial decarboxylases) into biogenic amines (Innocente & D'Agostin 2002). Ripening

cheeses are the next (after fish) most commonly implicated food item associated with biogenic amine poisoning (Stratton *et al*. 1991).

Quantitatively and toxicologically the most important biogenic amines (histamine, tyramine, tryptamine, phenylethylamine, cadaverine) in ripening cheeses are tyramine and histamine. Tyramine is a potent vasoconstrictor; its higher levels in an organism can lead to hypertension

and migraine and can induce brain haemorrhage and heart failure (Til *et al.* 1997). Histamine (also vasoactive substance) can cause urticaria, hypotension, headache, flushing, and abdominal cramps (Stratton *et al.* 1991). Tyramine and histamine are broken down in the mammalian organism by oxidative deamination catalysed by monoamine oxidase (MAO). However, human detoxification mechanisms are insufficient in the following cases: too high intake in a diet; in the allergic individuals; in patients consuming drugs acting as MAO inhibitors (antiparkinsonian drugs and antidepressants).

Within the group of biogenic amines, some polyamines (putrescine, spermidine and spermine; but not cadaverine, from the chemical viewpoint also a polyamine) are currently classified as a distinct group for the following reasons. Putrescine, and subsequently its higher metabolites spermidine and spermine, are synthesised in a mammalian cell from ornithine and therefore are already present in the raw materials (cheese milk; Coleman *et al.* 2004). Toxicological importance of polyamines is based on their ability to form stable carcinogenic N-nitroso compounds and to enhance the growth of chemically induced aberrant crypt foci in the intestine (Paulsen *et al.* 1997). Polyamines are required for normal cell growth and proliferation, but are readily taken up by tumor cells; a strict control of the polyamine content in the diet of the cancer patients is therefore a matter of the utmost importance (Kalač & Krausová 2005). Putrescine stimulates tyrosine kinases and the expression of particular nuclear protooncogenes and is in this sense involved in cancer pathogenesis (Wolter *et al.* 2004).

Apart from the milk pasteurisation (or the lack of it; Novella-Rodríguez *et al.* 2003), general hygienic conditions of the cheese production (Komprda *et al.* 2007) and starter culture (Roig-Sagués *et al.* 2002), the following factors can influence biogenic amine content in ripening cheese: the time of ripening (Komprda *et al.* 2007) and part of the cheese (Novella-Rodríguez *et al.* 2003; Komprda *et al.* 2007). The data regarding the assessment of these factors in blue-vein cheeses (Novella-Rodríguez *et al.* 2003) are surprisingly scarce. Therefore, the objective of the present study was to evaluate the biogenic amine and polyamine contents in one of the blue-vein cheese varieties from the viewpoint of the distribution within the cheese and of the dependence on the time of ripening.

MATERIALS AND METHODS

Cheese-making. The samples of the blue-veined cheese Niva were prepared in a production plant under the standard technological and hygienic conditions. The basic raw materials for the cheeses production were pasteurised full-fat cow milk, the mold *Penicillium roqueforti*, rennet, butter starter culture (*Lactococcus lactis*, subsp. *lactis,* and *L. lactis*, subsp. *cremoris*), and common salt. Milk was pasteurised at 73°C for 30 seconds. Cheese milk in a vat was cooled to 29°C, and before renneting, $CaCl_2$ (15 g/100 l), starter culture (0.9%), and mould culture (commercial *P. roqueforti* spores) were added (unfortunately, the producer refused to communicate both the particular *P. roqueforti* strain and the amount inoculated per ml of milk). The time of renneting was 60 min, that of curd forming 110 minutes. NaCl was added into the curd in the amount of 8 kg per 1000 l of milk. Loaves weighing 2 kg, with a diameter of 15 cm, height 12 cm, and the final fat content of 52% in dry matter were produced. Cheeses were subsequently soaked in a salt solution (20% NaCl) for 48 h (final NaCl content in the cheese was 4.8%), let to dry, pierced, and transferred to the cheese cellar, where they ripened at the temperature of 8°C.

Altogether six vats of blue-veined cheese were produced, two vats in each of the three different time periods: October (Vats 1 and 2), November (Vats 3 and 4), and December (Vats 5 and 6). Within each vat, 12 loaves were produced. Three randomly chosen loaves were taken from each cheese vat after 7, 21, 35, and 49 days of ripening.

Amines determination. At each sampling, the loaf was cross-cut in the middle, and 100 g of the edge part (3 cm thick outer part of the cheese, designated as "E") and 100 g of the remaining core part (designated as "C") were taken, respectively.

The E and C samples were homogenised separately. 10 g of each sample was weighed into a 85 ml test tube, 0.5 ml of the internal standard (1,7-diaminoheptane; concentration 1 mg/ml) was added and the sample was extracted with 20 ml of 0.1M hydrochloric acid (HCl) for 2 min using a disintegrator Heidolph Diax 900 (Heidolph Instruments, Germany). The suspension was centrifuged at 755 × g at 4°C for 10 min (Hettich Universal 32R; Hettich, Germany). The supernatant was filtered through a paper filter and the solid residue was extracted once again as described above. The combined extracts were made up to 50 ml with

deionised water and filtered through a disposable nylon membrane filter (13 mm, 0.45 μm; Chromatography Research Supplies, Addison, USA).

The extract was derivatised with dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride, DCl). The derivatising agent was prepared by dissolving 5 mg of dansyl chloride in 1 ml of acetone (Sigma-Aldrich, St. Louis, USA). The derivatisation was performed as follows: 1 ml of the extract (or standard) was mixed with 0.5 ml of saturated Na_2CO_3 (pH adjusted to 11.2), 1 ml of the derivatising agent was added and the mixture was shaken for 1 min (MS2 Minishaker IKA; IKA Werke GmbH, Staufen, Germany). The derivatisation proceeded in the dark at 40°C for 1 hour. The amine derivatives were then extracted with diethylether (3 × 1 ml). The organic phase was evaporated to dryness under nitrogen and the solid residue was dissolved in 0.5 ml of acetonitrile (ACN). The solution was filtered through the nylon membrane filter 0.45 μm and a volume of 10 μl was injected onto the chromatographic column.

Dansylated amines were separated using a liquid chromatograph HP 1100 (Agilent Technologies, Wilmington, USA) consisting of a quarternary pump (G1311A), vacuum degasser (G1322A), automatic sampler (G1313A), and UV/VIS detector with variable wave-length (G1314A). The separation after DCl derivatisation was carried out using gradient elution with H_2O/ACN (time 0–23 min: H_2O 35–0%, ACN 65–100%) on the Zorbax Eclipse XDB C18 column (150 mm × 4.6 mm, particle size 5 μm) with the guard column Meta Guard ODS-2 (30 mm × 4.6 mm, particle size 5 μm) at the flow rate of 0.8 ml/min. Photometric UV/VIS detector was used at 254 nm. The concentration of amines in the sample was corrected based on the method of internal standard according to Komprda *et al.* (2007).

The separated dansylated amines were identified by comparing their retention times with those of the particular dansylated amine standards (all the amine standards were used as the respective hydrochlorides supplied by Sigma-Aldrich and subsequently derivatised in the same way as the amines proper), and their concentrations after DCl derivatisation were expressed in mg/kg of cheese.

Two groups of amines are distinguished in the following text: "biogenic amines" (histamine, tyramine, tryptamine, and cadaverine,) and "polyamines" (putrescine, spermidine, and spermine). Despite the fact that the latter substances traditionally belong to biogenic amines, they are currently classified as a distinct group based on their biological properties (not their chemical structure); for the same reason, cadaverine (chemically also polyamine) is not included in these biologically active polyamines.

Statistical evaluation. Biogenic amines and polyamines were determined in duplicates in each part (core, edge) of each loaf within each vat of the cheese. As far as the number of replications is concerned, despite the fact that the cheese vat is usually considered an experimental unit in the cheese technology, if more cheeses are produced from a given experimental unit, the loaves can be looked upon as sub-units (Hunter *et al.* 1997). Therefore, the experimental data measured were assessed from four different viewpoints, and, correspondingly, four different sets of data were evaluated based on the following number of measurements: comparison of the vats in a given time of ripening, 3 measurements ($n = 3$); comparison of all cheeses produced in a given month: 3 loaves × two vats ($n = 6$); comparison of the parts of cheeses: all core parts and all edge parts, respectively, from all loaves from all vats produced within all three months were taken as two particular sets ($n = 18$); dependence on the time of ripening: 3 loaves × 6 vats × 4 samplings ($n = 72$).

The Unistat package, version 4.53 (Unistat Ltd., London, England), was used for the calculation of the basic statistical characteristics, regressions (that is the dependences of particular amine or group of amines contents on the time of ripening), including significance testing of the linear and quadratic terms, respectively, and the differences between the sets of cheeses (vats; loaves) in the amine contents (one-way classification of the variance-ratio test, including Duncan's multiple range test). The percentage of total variability in the amine content explained by particular tested variability factor (vat, part of the loaf, time of ripening) was calculated as the percentage of the sum of squares belonging to the particular factor from the total sum of squares using multiple-way classification of the variance-ratio test.

The *P*-level was used in the tables, figures, and within the text in the usual meaning of the probability of error that is involved in accepting the observed results as valid. *R*-square value (R^2) was used (in Figure 1 and within the text) in the sense of the coefficient of determination, the measure of

the variability of the dependent variable around the regression line, that is the percentage of the explained variability from the original variability.

RESULTS AND DISCUSSION

The contents of biogenic amines and polyamines, respectively, were affected differently by the tested variability factors in the present experiment (Table 1). The differences between the vats, including the differences between the production periods (October × November × December), accounted for one third of the explained variability of the sum of biogenic amines, but nearly for two thirds in the case of the sum of polyamines. This finding might seem paradoxical, because the differences in the amine contents between various production periods are supposed to originate from different microbial contamination of the raw milk and/or the environment of the production plant, and biogenic amines are most likely formed by the contaminant bacteria possessing the amino acid-decarboxylase activities (Novella-Rodríguez *et al.* 2003), contrary to polyamines which are mainly synthesised in eukaryotic cells (in dairy cow in this case; Coleman *et al.* 2004).

The difference between the sum of biogenic amines (nearly half of explained variability) and the sum of polyamines (only 4% of explained variability; Table 1) was even greater as far as the time of ripening is concerned. The likely reason is the

known fact that the content of biogenic amines increases during cheese ripening (Komprda *et al.* 2007, 2008a), which was confirmed also in the present experiment, see below); the content of polyamines usually changes less conspicuously with increasing time of ripening (Komprda *et al.* 2008a). However, the percentages of the explained variability due to the time of ripening found in blue-vein cheese in the present experiment differ from the corresponding data of our previous experiment with a Dutch-type semi-hard cheese (Komprda *et al.* 2008a), where the time of ripening accounted for 73% of the explained variability both of the sum of biogenic amines and the sum of polyamines.

Moreover, despite the above clear trends concerning the sums of biogenic amines and polyamines, respectively, great differences existed between the individual amines regarding the percentages of the explained variability, and, as also apparent from Table 1, a greater part the of total variability remained unexplained by the factors tested in the present experiment.

As already mentioned above, the difference between biogenic amines and polyamines regarding the changes during ripening can be expected (Komprda *et al.* 2008a). In the present experiment (when the relationships for a given amine using all values irrespective of the vat or production period were calculated), the content of no polyamine, with the only exception of spermine,

Table 1. Effect of particular tested factors on biogenic amines and polyamines contents in blue-vein cheese Niva (general linear model of the multiple-way analysis of the variance ratio test)

Amine	% of explained variability by the tested factors			Residual variability
	vat	part of the cheese	time of ripening	
Tyramine	30	39	31	59
Histamine	14	55	31	84
Tryptamine	54	1	44	91
Cadaverine	36	18	46	85
Phenylethylamine	69	14	17	90
Sum of biogenic amines[1]	36	18	46	73
Putrescine	61	26	12	87
Spermidine	70	0	30	96
Spermine	28	33	39	84
Sum of polyamines[2]	62	34	4	90

[1]tyramine + histamine + tryptamine + cadaverine + phenylethylamine; [2]putrescine + spermidine + spermine

significantly changed during ripening. Spermine content (Y, mg/kg) even decreased ($P < 0.05$) with increasing time of ripening (X, days) according to the equation $Y = 7.7 – 0.11X$ ($R^2 = 0.06$). Even putrescine, polyamine that (apart from the pathway from ornithine functioning in mammalian cells) can be alternatively synthesised from arginine in some bacteria (potentially contaminating cheese milk; Ohnuma *et al.* 2005) did not change during ripening of blue-vein cheese in the present experiment, contrary to the Dutch-type semi-hard cheese used in our previous experiment (Komprda *et al.* 2008a), where putrescine significantly ($P < 0.01$), though slowly, increased with increasing time of ripening.

The content of tryptamine (which belongs to biogenic amines) also tended ($P > 0.05$) to decrease during ripening in the present experiment. On the other hand, the contents of all other biogenic amines tended to increase with increasing time of ripening; significant ($P < 0.01$) relationships are shown in Figure 1.

As far as tyramine is concerned (tyramine was quantitatively the most important biogenic amine in the present experiment, see below), it is interesting that the content of this amine did not change ($P > 0.05$) in the edge part of the cheese (contrary to the core samples, Figure 1) during ripening.

Based on the constant of the linear term (11.7; Figure 1), the tyramine content in the core samples increased seventeen-times more rapidly during ripening in the present experiment than the tyramine content in the Dutch-type semi-hard cheese in our previous experiment (Komprda *et al.* 2008a). It should be underlined that this difference regards only different tyramine distribution between the core and edge parts of the cheese, not the total tyramine content in the compared kinds of cheeses as a whole. The authors of the experiment cited (Komprda *et al.* 2008a) ascribed the substantially higher increase of the tyramine content during ripening in outer parts of the Edam cheese to higher counts of contaminant enterococci in this part of the cheese. Without microbiological analysis, we can only presume higher counts of tyrosine-decarboxylating microorganisms in the inner part as compared to the edge part of the blue-vein cheese in the present experiment.

As far as the possible explanation for the uneven tyramine distribution within the blue-vein cheese in the present experiment is concerned, the conclusions of several other research groups that focused on this question can be mentioned: thus Petridis and Steinhart (1996), Novella-Rodríguez *et al.* (2003), and Komprda *et al.* (2007) admitted in agreement that they had not been able to present
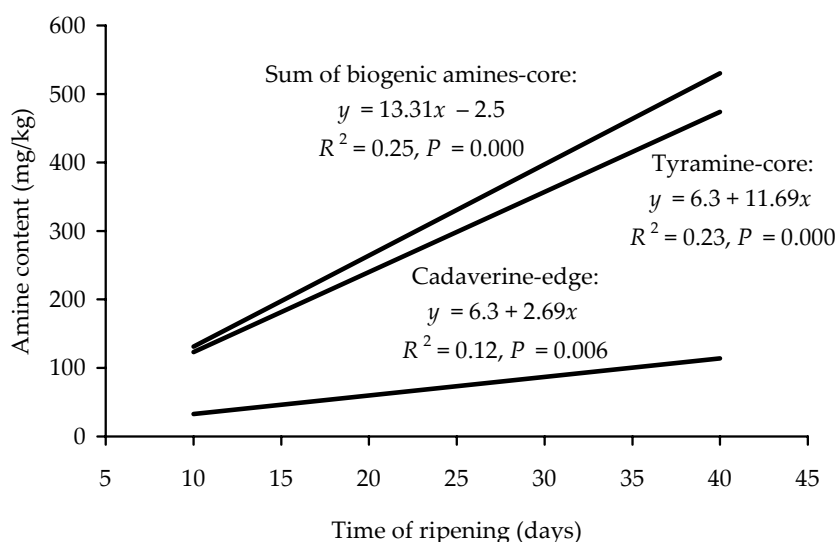

Figure 1. Dependence of biogenic amine content on the time of ripening (only significant relationships, $P < 0.01$, are presented); samples irrespective of vat or production period were taken as one set: $n = 72$ (3 loaves × 6 vats × 4 samplings); R^2 – coefficient of determination (interpretation: e.g. $R^2 = 0.25$ means that the variability of the amine content around the regression line is [1–0.25] times the original variance; in other words 25% of the original variability has been explained, 75% is residual variability)

any satisfactory explanation for their respective differences in tyramine distribution within the cheese, mentioning unspecified different external and internal microenvironmental conditions, possible different access of O_2 or different water activity (a_w). The latter factor ($a_{w'}$) was pursued in greater detail by KOMPRDA *et al.* (2008b), again with inconclusive results.

Apart from the dependences presented in Figure 1, the histamine content as well as the contents of the sum of all biogenic amines in the edge-samples, respectively, also significantly increased ($P < 0.05$) with increasing time of ripening ($Y = 8.4 + 0.49X$, $R^2 = 0.05$ and $Y = 66.1 + 4.43X$, $R^2 = 0.09$, respectively). The sum of all amines (biogenic amines + polyamines) increased much more rapidly in the core samples ($Y = 15.4 + 13.2X$, $R^2 = 0.25$, $P < 0.001$) in comparison with the edge ones ($Y = 87.3 + 4.48X$, $R^2 = 0.08$, $P < 0.05$).

All the results regarding the amines content in the cheeses are presented in fresh matter. We determined dry matter (DM) content, but only in mixed core/edge samples. Therefore, any differences between the amines contents in the core and edge parts of the cheeses due to the different DM content cannot be inferred from the results of the present experiment. On the other hand, the statements regarding the changes in biogenic amines contents during cheese ripening were not apparently influenced by this fact because the dry matter content did not change ($P > 0.05$) with increasing time of ripening (the mean of DM content of all cheese samples after 7 and 49 days of ripening was 56.8% and 57.4%, respectively).

As follows from Table 1, the factor of the vat affected substantially the contents of both biogenic amines and polyamines in the cheeses, this effect being partly caused by the time period in which the cheese was produced. However, when the three periods were directly compared, only the differences in the content of tyramine (quantitatively the most important biogenic amine) and in the sum of biogenic amines, respectively, between the cheeses produced in October, November, and December were demonstrated ($P < 0.01$), contrary to the sum of polyamines ($P > 0.05$; Figure 2). The variability due to the factors not tested in the present experiment likely outweighed the differences in the polyamine content (residual variability was 90% of total variability in this case, Table 1).

The cheese producer has used mixed milk from different suppliers and therefore it was not possible to evaluate during the experiment one important factor that can affect the amines content in the cheeses, namely the dairy cow's feed mixture composition. The months October to November can be the period of transition from the summer to the winter diet; the high silage content in the dairy cow's diet could introduce higher counts of different lactic acid bacteria into the cheese milk, including enterococci, proved tyramine producers in cheese (MARTUSCELLI *et al.* 2005).
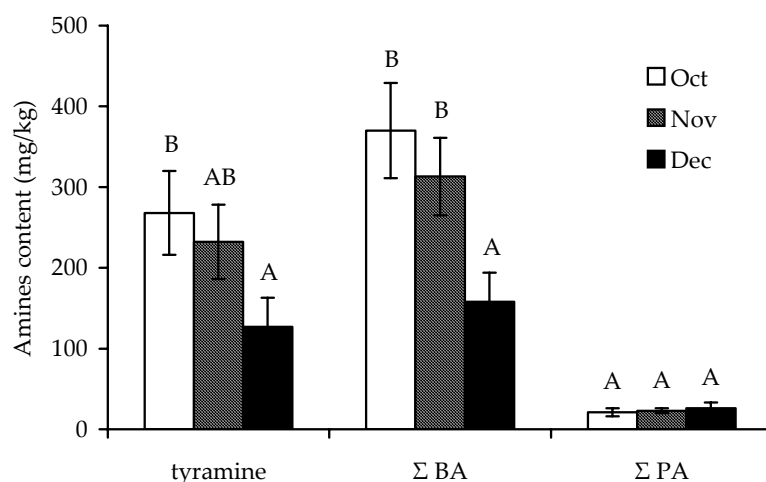

Figure 2. Effect of the production date (cheese produced in October, November and December, respectively) on the content of tyramine, sum of biogenic amines (Σ BA: tyramine + histamine + tryptamine + cadaverine + phenylethylamine) and polyamines (Σ PA: putrescine + spermidine + spermine), respectively; all loaves from both vats produced in a given month were taken as one set, $n = 6$; A, B – means with different superscripts differ at $P < 0.01$

The difference between biogenic amines and polyamines (Figure 2) can be also discussed from the following viewpoint. If it is true that putrescine (an essential precursor of the higher polyamines, spermidine and spermine) can be synthesised by alternative pathways from arginine in some bacteria, it is presumed that the substantial part of putrescine (and subsequently of spermidine and spermine) enters the milk via the mammalian (dairy cow in this case) putrescine synthesis pathway from ornithine (Coleman *et al.* 2004). The possible explanation for the same concentrations of the sum of polyamines in cheeses produced from milk originating in different months (Figure 2) may be the relatively constant rate of polyamine synthesis in a dairy cow. However, this explanation does not correspond with the data of Motyl *et al.* (1995), who found considerable variability in polyamine (spermidine and spermine) content in cow's milk due to the individual dairy cow, phase of lactation, milk yield, and age.

On the other hand, biogenic amine producers are mainly amino acid-decarboxylase positive strains within contaminant bacterial genera and species (Schneller *et al.* 1997; Öner *et al.* 2004). It can be suggested, based on the data of Figure 2, that the extent of secondary contamination by adventitious bacteria of the cheese milk or/and of the produced cheese differed substantially in various months in the present experiment. Bacterial counts were not determined in the present experiment, but based on our previous results (Komprda *et*

al. 2008b), the secondary contamination of the pasteurised milk by bacteria with proteolytic and/ or decarboxylating activities cannot be excluded regarding some vats produced within the present experiment.

As mentioned above, the main reason for the formation of biogenic amines in cheeses is the presence of microorganisms possessing decarboxylase activity (non-starter lactic acid bacteria, other spontaneous microflora, Roig-Sagués *et al.* 2002; starter microorganisms, Fernández-García *et al.* 2000). However, despite the fact that bacterial producers of biogenic amines in cheeses are well established: lactic acid bacteria (LAB), enterococci (in this regard an important group within LAB) and *Enterobacteriaceae* (Schneller *et al.* 1997; Roig-Sagués *et al.* 2002; Martuscelli *et al.* 2005), we found only either insignificant or even significantly negative correlations between biogenic amine content and the counts of above-mentioned bacteria in our previous experiment (Komprda *et al.* 2008b). In general, it is difficult to find direct correlation between microorganisms counts and biogenic amine content in cheese, because the amine-producing abilities of different strains of various bacteria differ widely (Valsamaki *et al.* 2000; Innocente & D'Agostin 2002). For these reasons, we resigned to the microbiological analysis in the present experiment. Similarly, one of the most distinguished research groups in the field (Novella-Rodríguez *et al.* 2003), reported the distribution of biogenic amines and polyamines
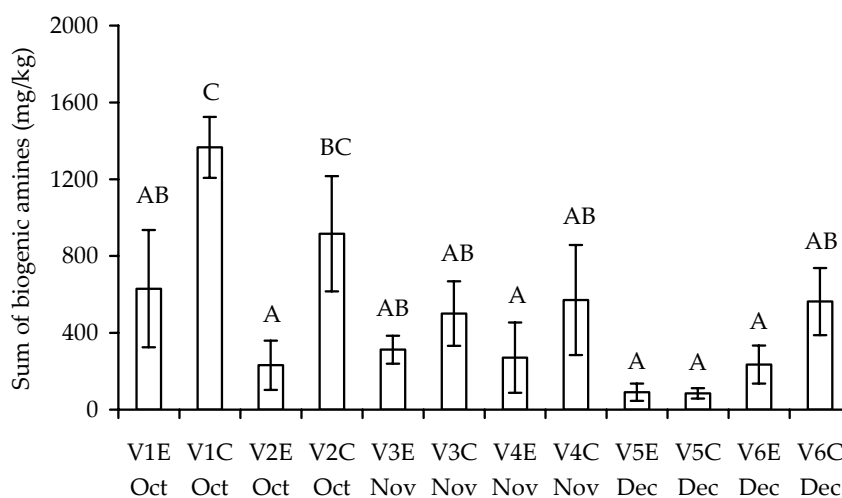
Figure 3. Sum of biogenic amines (tyramine + histamine + tryptamine + cadaverine + phenylethylamine) at the end of ripening (49 days) in the cheeses (vats; V1–V6) produced in October (Oct; V1, V2), November (Nov; V3, V4) and December (Dec; V5, V6); E – edge; C – core; *n* = 3; A, B, C – means with different letters differ at *P* < 0.01; *n* = 3
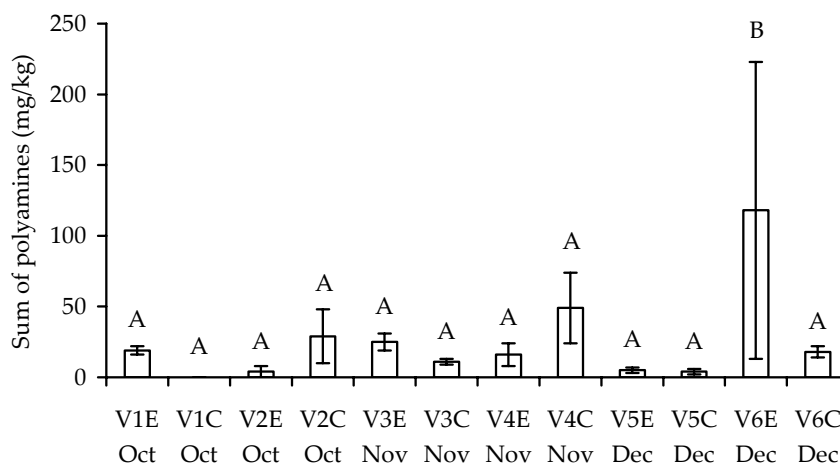
Figure 4. Sum of polyamines (putrescine + spermidine + spermine) at the end of ripening (49 days) in particular cheeses (vats; V1–V6) produced in October (Oct; V1, V2), November (Nov; V3, V4) and December (Dec; V5, V6); E – edge; C – core; n = 3; A, B – means with different letters differ at P < 0.01; n = 3

in various types of cheese without microbiological analysis.

The contents of individual biogenic amines and polyamines in the cheeses at the end of the experiment (after 7 weeks of ripening) are presented in Table 2; the sums of biogenic amines and polyamines at the end of ripening are shown in Figures 3 and 4, respectively.

It is apparent from Table 2 that, firstly, tyramine was quantitatively by far the most abundant amine (the mean and median being 380 mg/kg and 289 mg/kg, respectively) and, secondly, its content in different cheeses (vats) varied widely, from 10 mg/kg, to 875 mg/kg. Novella-Rodríguez *et al.* (2003) found the median of the tyramine content within 20 samples of blue cheeses obtained from Spanish retail stores to be 14 mg/kg with the range from not detected to 1585 mg/kg. Though the upper limits (875 mg/kg and 1585 mg/kg, respectively) are different, both values found in blue-vein cheeses are higher in comparison with the corresponding values in other kinds of ripening cheeses produced from pasteurised milk: Swiss-type (320 mg/kg; Petridis & Steinhart 1996) or Dutch-type (392 mg/kg; Komprda *et al.* 2008a). On the other hand, Roig-Sagués *et al.* (2002) reported maximum value of tyramine within the set of 44 samples of soft, semi-soft and hard traditional Spanish cheeses to be 1807 mg/kg; however, it is not apparent from the paper cited whether or not some cheeses were produced from unpasteurised milk.

Due to the great differences between people regarding the robustness of their detoxification system and possible synergistic effects of other biogenic amines, the toxicological limit for tyramine is difficult to establish. Among very different data having been published on the topic to date, the value of 100 mg/kg of food suggested in the review article of Silla-Santos (1996) is one of the more commonly used upper limits. Nine cheese samples from twelve tested in the present experiment (75%) can be considered unsafe from this viewpoint.

The median value of the histamine content (14 mg/kg; based on the figures in Table 2) in the blue cheese in the present experiment is comparable with the data of Novella-Rodríguez *et al.* (2003) regarding this type of cheese (7 mg/kg). However, the range of histamine values was much broader in the experiment by Novella-Rodríguez *et al.* (2003) (from not detected to 377 mg/kg) than in the present experiment (14–62 mg/kg), likely due to the fact that the cited authors evaluated cheese samples obtained from retail stores, presumably from different producers. In the present experiment, we used samples of blue cheese from only one producer (though the cheeses were produced in three different months). The histamine content exceeded 30 mg/kg in only two cheeses produced in October (Table 2). At any rate, no cheese tested in the present experiment exceeded the suggested (Silla-Santos *et al.* 1996) toxicological limit of 100 mg/kg.

Cadaverine was the second most abundant amine in the present experiment. Its concentration, simi-

Table 2. Biogenic amine and polyamine contents (mg/kg) in blue-vein cheese Niva at the end of ripening (49 days); mean ± standard error of the mean; $n = 3$

Amine	Cheese produced in											
	October				November				December			
	vat 1		vat 2		vat 3		vat 4		vat 5		vat 6	
	edge	core	edge	core	edge	core	edge	core	edge	core	edge	core
Tyr[1]	411[ab] ± 313	875[b] ± 328	166[ab] ± 110	849[b] ± 367	66[a] ± 14	452[ab] ± 178	153[ab] ± 62	823[b] ± 22	10[a] ± 2	58[a] ± 39	158[ab] ± 65	553[ab] ± 178
His[2]	90[b] ± 48	0[a] ± 0	21[ab] ± 11	59[ab] ± 28	25[ab] ± 5	8[ab] ± 4	19[ab] ± 4	0[a] ± 0	14[ab] ± 3	0[a] ± 0	13[ab] ± 1	0[ab] ± 0
Try[3]	0[a] ± 0	0[a] ± 0	1[a] ± 1	6[a] ± 5	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0
Cad[4]	132[a] ± 63	491[a] ± 285	45[a] ± 22	3[a] ± 2	222[a] ± 60	41[a] ± 9	235[a] ± 147	35[a] ± 5	68[a] ± 48	24[a] ± 19	66[a] ± 36	10[a] ± 5
Phe[5]	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0	0[a] ± 0
Put[6]	9[a] ± 4	0[a] ± 0	0[a] ± 0	0[a] ± 0	13[a] ± 6	0[a] ± 0	24[a] ± 0	44[a] ± 33	3[a] ± 2	0[a] ± 0	117[b] ± 105	12[a] ± 1
Spd[7]	4[a] ± 3	0[a] ± 0	0[a] ± 0	29[b] ± 19	0[a] ± 0	8[a] ± 0	0[a] ± 0	18[ab] ± 17	0[a] ± 0	4[a] ± 2	0[a] ± 0	6[a] ± 2
Spm[8]	7[ab] ± 3	0[a] ± 0	4[ab] ± 3	0[a] ± 0	12[b] ± 2	3[a] ± 2	0[a] ± 0	11[a] ± 8	2[a] ± 1	0[a] ± 0	1[a] ± 1	0[a] ± 0
ΣBA+ ΣPA[9]	649[ab] ± 308	1366[c] ± 159	236[a] ± 128	946[bc] ± 280	339[ab] ± 79	512[ab] ± 165	287[ab] ± 189	620[ab] ± 310	97[a] ± 47	89[a] ± 28	355[ab] ± 199	581[ab] ± 174

[1]tyramine; [2]histamine; [3]tryptamine; [4]cadaverine; [5]phenylethylamine; [6]putrescine; [7]spermidine; [8]spermine; [9]sum of biogenic amines (tyramine + histamine + tryptamine + cadaverine + phenylethylamine) + sum of polyamines (putrescine + spermidine + spermine)

a, b, c – means with different letters differ at $P < 0.01$

larly to that of tyramine, varied widely: between 3 mg/kg and 491 mg/kg (the mean 114, median 56 mg/kg). It follows from the data of Novella-Rodríguez *et al.* (2003) that, despite the relatively low median value 11 mg/kg, cadaverine can reach very high values in blue-vein cheese: more than 2100 mg/kg.

The level of other biogenic amines were very low in the present experiment, even at the end of ripening (Table 2).

The same was true regarding polyamines, with the exception of putrescine in the edge part of the vat 6-cheeses: 117 mg/kg (Table 2). The same putrescine concentration was found already in the vat 6-edge sample during the initial sampling (7th day of ripening). Putrescine (polyamines) can be synthesised both in mammalian tissues (their biosynthesis being regulated mainly by the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase; Hillary and Pegg 2003) and in microorganisms (alternative pathway involving l-arginine decarboxylase, which was not confirmed in mammals; Coleman *et al.* 2004). Considering the fact that the putrescine content in fresh milk is usually very low, its atypically high level (117 mg/kg) can be indicative of some hygienic error during the milk treatment before renneting.

The median of the putrescine content in the present experiment, 6 mg/kg (the mean being 18 mg/kg), is comparable with the data of (Novella-Rodríguez *et al.* 2003) for Spanish blue cheeses, 18 mg/kg. However, the quoted authors (Novella-Rodríguez *et al.* 2003) reported more than twice as high upper level of putrescine content (257 mg/kg) than was that found in the present experiment (117 mg/kg). The mean values of not only putrescine content, but also of spermidine and spermine contents found in the present experiment resemble the corresponding means measured in two varieties of Norwegian blue cheese, Saga and Normanna, in an experiment by Eliassen *et al.* (2002): 12 and 16, 15 and 24, and 2 and 0.4 mg/kg, respectively. Novella-Rodríguez *et al.* (2003) reported the highest (*P* < 0.05) spermidine content in blue cheese in comparison with all other tested unripened and ripened semi-hard and hard cheeses tested. The authors explained it by the known fact that mold cells can contain higher levels of polyamines. This could be the case of the *Penicillium roqueforti* mold in the experiment cited, but obviously not in the present experiment.

The several times higher (*P* < 0.01) polyamines content in the P6-edge sample in comparison with that in the P6-core sample (Figure 4; it seems paradoxical from the above viewpoint, because *Penicillium roqueforti* spores are supposed to be present predominantly inside the cheese) is indicative of some other polyamine producers, possibly contaminant bacteria possessing enzyme equipment for a parallel polyamine biosynthesis pathway from arginine, via agmatine (not measured in the present experiment) as an intermediate (Sekowska *et al.* 1998).

As far as the comparison with other cheese varieties is concerned, it is interesting (regarding the data shown in Table 2) that we found very similar putrescine distribution among 16 different samples of the Dutch-type semi-hard cheese in our previous experiment (Komprda *et al.* 2008a): most samples below 40 mg/kg, and only one (significantly different) value slightly above 120 mg/kg.

Generally speaking, the content of polyamines was very low in most samples in the present experiment and neither harmful nor favourable (wound healing) effects on the consumers can be expected. The no-observed-adverse-effect levels (a measure of the chronic toxicity) for putrescine, spermidine, and spermine were reported to be 180, 83, and 19 mg/kg body weight per day, respectively (Til *et al.* 1997). Similarly, no harmful effect, due to the consumption of the blue cheese evaluated in the present experiment can be expected in cancer patients where the polyamine intake should be restricted (Eliassen *et al.* 2002).

However, polyamines content measured in the cited papers and in the present experiment cannot be properly evaluated before both the recommended dietary intake of polyamines (growth and development of the digestive system, wound healing) and the upper limit regarding cancer patients has been established by further research (Komprda *et al.* 2008a).

The sum of all amines measured (biogenic amines + polyamines) was in the range of 89 mg/kg (core part of one of the December cheeses) to 1366 mg/kg (core sample of one of the October cheeses) in the present experiment. According to the review of Silla-Santos (1996), the amine level of 1000 mg/kg food is considered dangerous for health. However, the toxicological level of amines is difficult to establish because it depends on individual characteristics and the presence of other amines. The synergistic effects are described with histamine and

other amines (Silla-Santos 1996). However, this was not the case in the present experiment, where the above-mentioned upper value (1366 mg/kg) concerned exclusively tyramine and cadaverine (Table 2). In any rate, if not the healthy consumers, then the patients consuming monoamine oxidase inhibitors should avoid the consumption of such a product.

The values of the sum of biogenic amines at the end of ripening (Figure 3) reflect tyramine and cadaverine contents shown in Table 2. Moreover, the trend of higher biogenic amine content in the core samples in comparison with the edge ones is apparent from Figure 3; the differences were significant (P < 0.01) in the cheeses produced in October.

As far as polyamines are concerned, the content of the sum of all polyamines at the end of ripening did not differ ($P > 0.05$) between cheeses, with the exception of the December vat 6-edge samples (Figure 4) that had a higher ($P < 0.01$) sum of polyamines content in comparison with all other cheeses. This was the consequence of the unusually high putrescine content in this cheese (117 mg/kg; Table 2).

The higher biogenic amine contents in the core parts of the cheeses as compared to those in the edge parts (Figure 3) reflect substantially higher tyramine contents in the C-samples and are rather deceptive as far as the comparison of the other

individual amines is concerned. As follows from Figure 5, a higher ($P < 0.01$) content in the core-samples was found only in the case of tyramine. The concentrations of histamine, cadaverine, putrescine, and spermine at the end of ripening (after 49 days of ripening), were higher ($P < 0.01$) in the edge part of the cheese in comparison with the core samples, when all cheeses within all vats produced within all three intervals (October + November + December) were taken as one set.

The different distribution of polyamines (putrescine and spermine) within the cheese in the present experiment (Figure 5) disagrees with the findings by Novella-Rodríguez *et al.* (2003), who reported no difference in the polyamine content in relation to the part of the ripened semi-hard and hard cheese produced from goat milk, which the authors viewed as a reinforcement of the non-microbial origin of these substances.

As far as the distribution of tyramine is concerned, the results of the present experiment contributed to the inconsistence of the data reported in the available literature. Novella-Rodríguez *et al.* (2003) found much higher tyramine content inside ripened goat milk hard cheese than in the edge. The authors suggested that tyramine producers likely prefer anaerobic conditions prevailing in the internal parts of cheese. On the other hand, the tyramine content was higher in he outer parts of
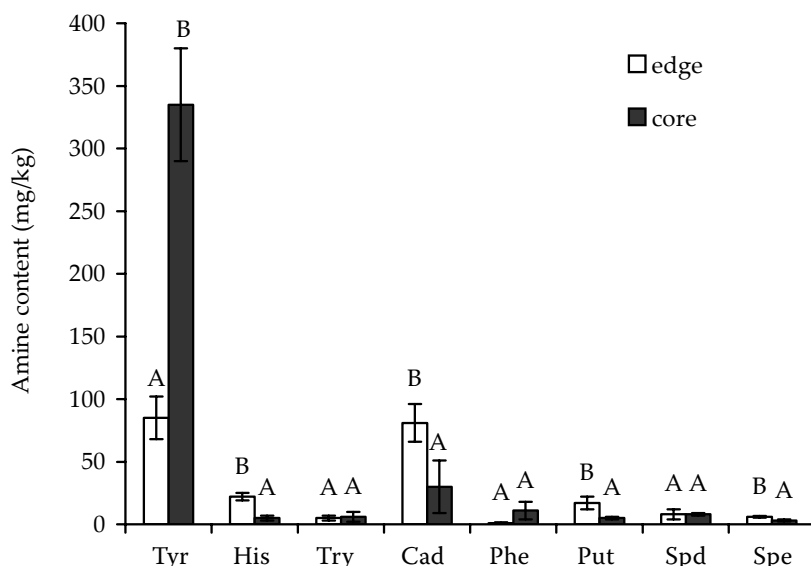

Figure 5. Comparison of the core and edge parts of the cheese at the end of ripening (after 49 days of ripening) regarding the content of particular biogenic amines and polyamines; all loaves from all vats produced within the whole production interval October–December were taken as one set, *n* = 18; A, B – means with different letters differ at *P* < 0.01; Tyr – tyramine, His – histamine, Try – tryptamine, Cad – cadaverine, Phe – phenylethylamine, Put – putrescine, Spd – spermidine, Spe – spermine

both the Swiss-type cheese (Petridis & Steinhart 1996) and the Dutch-type semi-hard cheese in an experiment by Komprda *et al.* (2008a).

The last-mentioned authors attributed this finding to the secondary enterococci contamination during the cheese production and/or to different environmental conditions in the cheese (counts of enterococci, which belong to the confirmed tyramine producers in cheese, were significantly higher in the outer parts of the cheese). To screen various *Penicillium roqueforti* strains from the viewpoint of their putative potential to decarboxylate tyrosine can be viewed as an objective for further research.

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