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Contents of biogenic amines and microbiota in typical slowak cheeses

Gehalte an biogenen Aminen und Mikroflora in typischen slowakischen Käsesorten

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Summary

We analyzed 25 cheeses from the Slovak market for their contents of biogenic amines and polyamines, microbiota, pH and water activity. Contents of tryptamine were below the limit of detection (4.2 mg/kg) in 24 samples. For the other amines, contents varied considerably between samples, with lowest values below the limit of detection and maximum contents of 1117.5; 155.0; 52.7; 296.2; 157.0; 27.6; 49.1 mg/kg fresh matter for ß-phenylethylamine (PHE), putrescine, cadaverine, histamine, tyramine, spermidine and spermine, respectively. Histamine contents >100 mg/kg were detected in 2 semi-dry- and 1 dry-cheese sample. The amount of PHE ingested by a 60 or 200 g cheese portion would exceed the lowest-observed-adverse-effect-level of 5 mg (single dose per consumer) in 6/25 and 11/25 samples, respectively. No sample exceeded food safety limits for *Salmonella* and *Listeria monocytogenes*. Likewise, contents of coagulase-positive Staphylococci were consistently < 1 log cfu/g. Considerable contents of Enterobacteriaceae (6.9 log cfu/g) and *E. coli* (3.9 log cfu/g) were detected in one fresh cheese made from unpasteurized milk. High bacterial numbers were, in general, not associated with higher biogenic amine contents.

Keywords: cheese, histamine, ß-phenylethylamine, alimentary exposure

Zusammenfassung

In 25 Käseproben des slowakischen Marktes wurden Gehalte an biogenen Aminen und Polyaminen, Mikroflora, pH-Wert sowie die Wasseraktivität bestimmt. Die Tryptamingehalte waren bei 24 Proben unter der Nachweisgrenze von 4,2 mg/kg. Die Gehalte der anderen Amine schwankten stark, mit niedrigsten Werten unter der Nachweisgrenze und Höchstwerten von 1117,5; 155,0; 52,7; 296.2; 157,0; 27.6; 49,1 mg/kg Frischmasse für ß-Phenylethylamin (PHE), Putrescin, Cadaverin, Histamin, Tyramin, Spermidin und Spermin. Histamingehalte >100 mg/kg wurden in 3 schnittfesten Käsen nachgewiesen. Die über eine 60 bzw. 200 g Käseportion aufgenommene PHE Menge würde den lowest-observed-adverse-effect level von 5 mg/Portion und Person bei 6/25 und 11/25 Proben überschreiten. Lebensmittelsicherheitskriterien für *Salmonella* und *Listeria monocytogenes* wurden bei keiner Probe überschritten. Ebenso waren die Gehalte an Koagulase-positiven Staphylokokken immer < 1 log_{10} KBE/g. Hohe Enterobacteriaceen- (6.9 log_{10} KBE/g) und *E. coli*- (3.9 log_{10} KBE/g) Konzentrationen wurden in einer Frischkäseprobe aus Rohmilch nachgewiesen. Hohe Bakterienzahlen waren im Allgemeinen nicht mit hohen Amingehalten assoziiert.

Schlüsselwörter: Käse, Histamin, ß-Phenylethylamin, alimentäre Exposition

Introduction

Milk and dairy products form an important part of human nutrition. Whereas the consumption of milk and cheese in Slovakia decreased from 1998–2003 (Jamborova et al., 2005), a rise was observed in the following periods, e. g. from 153 kg in 2008 per head and year to 158.6 kg in 2012 (Krizova and Budai, 2015). As in other countries, the cheese varieties produced and consumed in Slovakia range from fresh to hard cheeses with different fat contents. Currently, six Slovak cheese products have the "Protected Geographical Indication" (PGI) status under EU Regulation No. 1151/2012, amongst which are "bryndza", a soft/spreadable fresh cheese, "parenica", a cylinder-shaped pasta-filata type of cheese, and "ostiepok", a cheese of egg-shaped form with ornaments. In a survey from 2008, roughly 2/3 of 600 interviewed Slovak consumers were familiar with the term PGI, and associated higher quality with PGI foods. However, less than 50 % of the panel knew Slovak cheeses with PGI status (Supekova et al., 2008). This may, however, have changed in the last nine years.

Slovak cheese with the longest tradition was originally made from sheep's milk (Laukova and Czikkova, 2001). The best known are bryndza and steamed cheese (i. e. sorts where the cheese obtains its characteristic structure and properties during a heating-stretching process; Zimanova et al., 2016), in particular, ostiepok and parenica.

At present, Slovak bryndza contains at least 50 % of sheep's milk. Other types of Slovak cheeses are made mainly from cow's milk (Keresteš, 2007).

The microflora of cheeses varies among the different cheese types but in principle is dominated by lactic acid bacteria, enterococci and occasionally yeasts and fungi (Krömker, 2006). Bacteria may originate from the milk (if raw milk is used), the milking and processing environment or they have been deliberately added as "starter cultures", and the evolution of this flora is mainly influenced by salt content/drying, acidification, temperature and antagonistic bacterial effects. European Union legislation on microbiological criteria for foods currently includes four criteria applicable for cheeses, viz. food safety criteria for *Listeria monocytogenes, Salmonella* and process hygiene criteria for *E. coli* and coagulase-positive Staphylococci (Reg. (EC) No. 2073/2005).

In addition to bacteria, some protein degradation products are relevance for food safety. Biogenic amines are nitrogenous compounds originating from the decarboxylation of amino acids, whereas the term "polyamines" is reserved for synthetized compounds, and certain precursors (putrescine). Some representatives of the former group are involved in allergy and inflammation; they may decrease (histamine) or elevate (tyramine, tryptamine) blood pressure (Beutling, 1996) and their accumulation in proteinaceous foods indicates decomposition of proteins to amino acids, during either ripening or spoilage, and subsequent utilization of these compounds by bacteria. Polyamines are involved in cell regeneration and growth, thus high contents can be expected in metabolically highly active tissues/cells (e. g., Villanueva-Valero et al., 2005). Cheese is amongst the proteinaceous foods with a potential for high amine contents (Stratton et al., 1991; Linares et al., 2011). The ingestion of foods with higher amine levels has been associated with foodborne illness, with allergy-like or cardiovascular symptoms (Beutling, 1996). The health significance of alimentary exposure to biogenic amines is acknowledged in food safety legislation as regards histamine in fish (Reg. (EC) No. 2073/2005). More recently, alimentary exposure has been evaluated by EFSA (2011).

As regards Slovak cheese products, a number of studies have dealt with the microflora, esp. of the bryndza cheese type (Laukova and Czikkova, 2001; Jurkovič et al., 2007; Laurenčík et al., 2008; Berta et al., 2009; Chebeňová-Turcovská et al., 2011; Belicová et al., 2013; Pangallo et al., 2014; Planý et al., 2016), but scarce information exists on the contents of biogenic amines and polyamines in such products. In order to provide such information, we analyzed 25 cheeses from the Slovak market for their contents of biogenic amines and polyamines, microbiota, pH and water activity. Contents of biogenic amines were related to typical portion sizes in order to assess if consumers' exposure to biogenic amines would exceed threshold levels. The threshold levels and limits we used in this study were taken from series of assessments based on consumers' consumption habits in Austria (Rauscher-Gabernig et al., 2009, 2010, 2012; Paulsen et al., 2012; Wüst et al., 2017). In addition, some basic characterization of the microflora was done.

Materials and Methods

Obtaining cheese samples

Cheese samples were bought in supermarkets in/around Košice, Slovak Republic, in September 2016. Each sample was one original package, and was transported under refrigeration and stored at +2 °C until examination.

Determination of biogenic amines

10.0 g of finely chopped cheese mass were homogenized with 90 ml 10 % trichloroacetic acid for 2 min (Ultra Turrax T25; Jahnke&Kunkel, D). The suspension was filtered through a folded filter (MN 615¼; Macherey-Nagel, Düren, D) and further purified by a 0.2 µm cellulose acetate syringe filter (Roth, Karlsruhe, D). To 0.5 ml of this filtrate, 1 ml of dansylchloride solution (5 mg dansylchloride/ml acetone) and 1 ml saturated sodium hydrogencarbonate solution were added and the mixture was incubated in a closed vessel for 10 min. at 70 °C, and then taken to dryness at 40 °C under reduced pressure (Büchi Rotavapor R-100, Fläwil, CH). The dry residue was dissolved in 2 ml acetonitrile. After centrifugation at 12000 x g, 45 min at 5 °C (Sigma 3K30, Osterode a.H., D), an aliquot of the supernatant was used for analysis of the amine contents. Separation of danyslated amines was performed by HPLC (Waters Alliance 2695, Waters, Milford, USA) on a RP-C18 column (ACE RP-18, 3 µm, 125 x 3 mm, Advanced Chromatography Technologies, Aberdeen, UK), and detection was done by UV absorption at 252 nm (photodiodearray detector Waters 996). Amine standards (tryptamine, ß-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine) were dissolved in 0.1 n HCl and processed with dansylchloride and sodium hydrogencarbonate as described above. Identification of amines was done by comparing retention times of sample peaks to those of the known standards. Quantification was done according the external standard method. Reagents were obtained from Merck (Merck, Darmstadt, D), with exception of amine standards and dansylchloride (Sigma Aldrich, St. Louis, USA). Elution was performed by an acetonitrile – methanol – 0.02 n acetic acid gradient (Paulsen et al., 1997). Eluents were obtained from Chem-Lab (Zedelgem, B). For tryptamine, limit of detection (LOD) was 4.2

mg/kg; other LODs are indicated in Table 2. For calculations of the relations of amines to bacterial numbers, pH, and water activity, results below the limit of detection were set to ½ limit of detection ("middle bound").

Physicochemical measurements

Cheese samples were allowed to reach room temperature. Water activity was determined from finely ground cheese mass in a portable Novasina Lab-Swift aW meter (Novasina, Lachen, CH), whereas pH was determined in cheese directly by means of a penetrating electrode (Mettler-Toledo, Greifensee, CH) attached to a Testo 230 pH meter (Testo AG, Lenzkirch, D). Measurements were carried out in duplicate and the average is reported as the final result.

Microbiological examination

An aliquot of 25 g sample was suspended in 225 g sterile 0.85 % saline, and the suspension was homogenized in a stomacher blender for 180 sec. (Interscience, St. Nom, F). Subsequently, decimal dilutions were prepared in saline. 0.1 ml aliquots of these dilutions were spread onto Plate Count Agar (PCA; aerobic incubation for 72 h at 30 °C), Violet-Red-Bile-Glucose-Agar (aerobic incubation for 24 h at 37 °C), MRS Agar (aerobic incubation for 72 h at 30 °C) and a 1.0 ml aliquot was distributed on 3 plates of RPF-Baird-Parker Agar (aerobic incubation for 48 h at 37 °C) for determination of total aerobic counts (TAC), Enterobacteriaceae (EB), lactic acid bacteria (LAB) and staphylococci, respectively. Presumptive Enterobacteriaceae were confirmed by oxidase testing of subcultures on PCA. For enumeration of *E. coli,* 1.0 ml aliquots were inoculated via pour-plate technique into Coli-ID agar. Testing for *Salmonella* and *Listeria monocytogenes* was done from 25 g – enrichment cultures via immunoassays (Vidas SLM II and LMO2; Bio Merieux, Marcy l' Etoile, F), with protocols and reagents according to manufacturer's specifications. Limit of detection was 2 log cfu/g for TAC, LAB, EB and 1 log cfu/g for Staphylococci and *E. coli.* Media were obtained from Merck, with exception of Coli-ID and Vidas Reagents (Biomerieux, F). Results below limit of detection were set to "9" for "<10 cfu/g" and "99" for "<100 cfu/g" ("upper bound"). Bacterial numbers were then transformed to log counts.

Statistical processing of data

Besides descriptive statistics, correlations between bacterial numbers with pH, water activity and bacterial numbers were tested by Pearson's correlation analysis. Statistical significance was established at P≤0.05.

Results and discussion

Characterization of samples

In total, 25 cheeses were examined, of which 19 were of semi-dry type. Most of these cheeses (11 of 19) had been smoked. One sample was a dry cheese, and the remaining 5 were of fresh to semifresh type (Tab. 1). All samples were examined before or at the "best-before" date.

Contents and assessment of biogenic amines contents in the cheese samples

Contents of tryptamine were below the limit of detection in 24 samples, in the remaining single sample, 29.4 mg tryptamine /kg were determined. For the other amines, contents varied considerably between samples, with lowest values below the limit of detection and maximum contents of 1117.5; 155.0; 52.7; 296.2; 157.0; 27.6; 49.1 mg/kg for ß-phenylethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine, tyramine (TYR), spermidine and spermine, respectively (Tab. 2).

PHE levels in dairy products may exceed 100 mg/kg (Rauscher-Gabernig et al., 2010) or, in extreme cases, even 300 mg/kg (Linares et al., 2012), but in most samples, they will be well below 25 mg/kg. A somewhat different situation was encountered in our samples, where 17/25 samples had PHE contents >25 mg/kg. Rauscher-Gabernig et al. (2010) derived a lowest-observed-adverse-effect-level (LOAEL) of 5 mg per portion/single ingestion. We related this LOAEL to the median (60 g/day) and the 95th percentile

TABLE 1: *Description of samples.*

No.	Designation	Classification	Surface	Packing
1	Bryndza ovčia	full-fat fresh soft cheese – bryndza; non-pasteurized sheep milk		wrapped
2	Ovčí hrudkový syr	full-fat fresh soft cheese; non-pasteurized sheep milk		wrapped
3	Ovčí syr údený	full-fat soft cheese; pasteurized sheep milk	smoked	vac
4	Bánovecký pološtiepok neúdený	Steamed unripened semi-soft, semi-skimmed		map
5	Bánovecké korbáčiky neúdené	Steamed unripened semi-soft, semi-skimmed		map
6	Hravé korbáčiky	Semi-hard unripened mid-fat steamed cheese	smoked	map
7	Hravé pareničky	Semi-hard unripened mid-fat steamed cheese	smoked	map
8	Hravé syrčeky	Semi-hard unripened mid-fat steamed cheese	smoked	map
9	Parenica údená	Semi-hard unripened mid-fat steamed cheese	smoked	map
10	Pološtiepok údený	Semi-hard unripened full-fat steamed cheese	smoked	map
11	Syrové uzlíky údené	Semi-hard unripened mid-fat salty steamed cheese	smoked	vac
12	Syrový korbáč údený slaný	Semi-hard unripened mid-fat salty steamed cheese	smoked	map
13	Gazdovská chut'ovka bylinková	Semi-hard unripened mid-fat salty steamed cheese, non-pasteurized		vac
14	Zázrivský slimáčik gazdovský	Semi-hard unripened mid-fat salty steamed cheese; non-pasteurized		vac
15	Parenica neúdená	Semi-hard unripened mid-fat steamed cheese		map
16	Gazdovské uzlíky paprika pikant	Semi-hard unripened mid-fat salty steamed cheese, non-pasteurized		vac
17	Strúhaný liptovský výber	Semi-hard ripened half-fat grated cheese		map
18	Oštiepok údený	natural semi-hard mid-fat cheese	smoked	vac
19	Eidam údené plátky	Semi-hard ripened mid-fat cheese	smoked	map
20	Tekovský neúdený	Semi-hard ripened full-fat cheese, PGI		map
21	Vrchár ovocný	Semi-hard ripened full-fat cheese		map
22	Tekovský údený	Semi-hard ripened full-fat cheese, PGI	smoked	map
23	Volovec plátky	natural full-fat semi-hard cheese matured under morter		map
24	Syrové uzlíky	Semi-hard unripened mid-fat salty steamed cheese, non-pasteurized		vac
25	Vrchár extra vyzretý	hard ripened full-fat cheese		map

map: modified atmosphere-packed; vac: vacuum-packed

(200 g/day) cheese consumption of a female consumer with 60 kg bodyweight (Elmadfa and Freisling, 2004), which resulted in permissible levels of 85 and 25 mg PHE/kg cheese (fresh matter). For 6/25 samples, even a 60 g portion would exceed the LOAEL; for 11 samples, a 200 g portion would exceed this limit. Long-term ripened cheeses may provide an adequate amount of the free precursor amino acid to allow for formation such such amounts of PHE (Bütikofer and Fuchs, 1997). Maximum tolerable contents for putrescine and cadaverine were based on a provisional-acceptable-daily-intake (PADI) of 0.6 mg/kg bodyweight/day for PUT and the toxicological threshold level of 1.8 mg/kg bodyweight/day for CAD (Rauscher-Gabernig et al., 2012). Tolerable concentrations were 600 and 180 mg PUT/kg, for 60 g and 200 g cheese portions, respectively. The corresponding limits for CAD were 1800 and 540 mg/kg. For TYR, a NOAEL of 200 mg per single oral administration was derived (Paulsen et al., 2012). This corresponds to 3330 mg/kg cheese and 60 g consumption, or 1000 mg/kg for 200 g cheese consumption. None of these limits was exceeded in our samples. Tolerance levels for histamine ranged from 417 to 100 mg/kg cheese (Rauscher-Gabernig et al., 2009). The upper limit was not exceeded; however, three samples exceeded the lower limit of 100 mg/kg.

As can be seen from Table 2, there were large variations in amine contents, which might be attributed to different processing technology, different milk quality in raw-milk-cheeses (Novella-Rodríguez et al., 2004), and specific strains in the contaminant microflora (Mayer and Fiechter, 2013). The latter two factors could not be accounted for in this study. Likewise, for portions from ripened cheeses, it was not known if these samples were from the core or the periphery of the cheese – which is known to affect amine contents (Novella-Rodríguez et al., 2003).

Water activity and pH of cheeses

Water activity ranged from 0.92 to 0.99, with a median of 0.96. As could be expected, the dry cheese sample had lowest a_W, whereas the highest water activity was measured in a fresh cheese sample (Tab. 2). Water activities around 0.99 and 0.91–0.92 for fresh and semi-dry/dry cheeses, respectively, are in the expected range (Krömker, 2006). As regards pH, both lowest (4.7) and highest value (6.2) was measured in fresh cheese samples. The 19 semi-dry cheeses clustered in the pH-range 5.2–5.4 (Tab. 3).

Microflora of cheeses

Salmonella and *Listeria monocytogenes* were not detected in 25 g-aliquots of any sample. Thus, samples fulfilled the food safety requirements given by Reg. (EC) No. 2073/2005. Staphylococci counts were below the limit of detection in 16/25 samples, and the maximum was 3.7 log cfu/g. Coagulase-positive staphylococci were not detected in any sample (i. e. <1 log cfu/g), which indicated compliance with EU process hygiene criteria. Numbers of Enterobacteriaceae were below the limit of detection in 21/25 samples, and the maximum was 6.9 log cfu/g. The corresponding figures for *E. coli* were 22/25, and 3.9 log cfu/g. The EU process hygiene criteria were exceeded in two samples, a semi-dry cheese with 3.3 log *E. coli*/g; and in a fresh cheese made from raw ewe's milk (3.9 log *E. coli*/g). Since the latter cheese was sold in an open plastic bag, it cannot be excluded that these Gram-negative bacteria were introduced at retail, and were not introduced during processing or were present in the milk used for cheese production.

Total aerobic counts reached up to 8.9 log cfu/g (Tab. 3). In 15 of 25 samples, TAC corresponded well with numbers of lactic acid bacteria (differences not exceeding 1 log unit).

TABLE 2: *Contents of biogenic amines and polyamines in 25 typical Slowak cheeses, mg/kg fresh matter.*

No.	ß-phenyl ethylamine	putrescine	cadaverine	histamine	tyramine	spermidine	spermine
1	<5.0	31.6	52.7	42.4	75.5	22.0	12.7
2	<5.0	18.9	26.4	21.1	19.7	16.5	18.5
3	<5.0	17.5	21.6	27.9	20.4	20.5	11.4
4	<5.0	17.4	<3.7	<3.9	19.0	18.2	<4.8
5	58.1*	20.5	22.5	40.3	<4.3	18.5	<4.8
6	43.7	19.7	<3.7	24.4	<4.3	18.5	<4.8
7	45.3	19.5	<3.7	25.9	<4.3	<5.1	<4.8
8	84.7	23.0	<3.7	33.0	<4.3	20.5	12.3
9	48.0	20.4	<3.7	30.6	<4.3	<5.1	<4.8
10	26.1	<3.2	<3.7	21.1	<4.3	<5.1	<4.8
11	<5.0	17.7	21.7	26.3	20.3	22.2	12.9
12	35.9	17.6	21.9	21.3	<4.3	23.0	11.3
13	38.2	19.4	25.1	23.6	24.7	27.6	18.9
14	<5.0	19.9	29.7	25.6	24.6	26.0	15.7
15	36.2	18.6	<3.7	24.5	19.2	20.0	<4.8
16	65.4	17.6	28.8	30.5	19.3	18.6	11.3
17	460.1	110.7	<3.7	58.0	<4.3	19.6	20.4
18	117.4	17.9	22.5	45.4	20.7	20.7	49.1
19	60.9	21.9	<3.7	47.5	22.6	23.5	41.8
20	<5.0	50.6	24.2	33.9	<4.3	24.5	31.1
21	741.7	122.8	<3.7	128.0	157.0	<5.1	16.0
22	47.0	<3.2	22.3	33.5	20.6	19.6	11.7
23	1117.5	155.0	<3.7	296.2	<4.3	<5.1	<4.8
24	<5.0	21.9	27.5	26.4	22.5	18.8	11.9
25	884.4	136.3	<3.7	211.3	39.4	<5.1	<4.8
Minimum	<5.0	<3.2	<3.7	<3.9	<4.3	<5.1	<4.8
Maximum	1117.5	155.0	52.7	296.2	157.0	27.6	49.1

*: light grey and dark grey fields indicate that the respective tresholds or tolerable limits (for single oral administration to an average consumer of 60 kg bodyweight) are exceeded by a 200 g or a 60 g portion, respectively. Sample designations see Table 1.

TABLE 3: *Bacterial numbers, pH and water activity in 25 typical Slowak cheeses.*

No.	total aerobic count, log cfu/g	Enterobacteriaceae/ *E. coli*, log cfu/g	lactic acid bacteria, log cfu/g	Staphylococci*, log cfu/g	pH	a_w
1	8.1	<2 / <1	8.0	2.6	4.7	0.99
2	8.3	6.9 / 3.9	8.5	<1	6.2	0.99
3	8.5	<2 / <1	8.1	<1	4.9	0.96
4	<2	<2 / <1	<2	<1	5.3	0.96
5	5.6	<2 / <1	5.4	<1	5.3	0.97
6	<2	<2 / <1	<2	<1	5.3	0.95
7	<2	<2 / <1	<2	<1	5.1	0.97
8	2.3	<2 / <1	<2	<1	5.2	0.97
9	2.5	<2 / <1	<2	<1	5.3	0.96
10	2.5	<2 / <1	<2	<1	5.3	0.96
11	3.3	<2 / <1	<2	<1	5.1	0.96
12	3.4	<2 / <1	<2	2.6	5.2	0.93
13	3.8	<2 / <1	<2	<1	5.2	0.96
14	4.3	<2 / <1	4.3	3.4	5.2	0.95
15	5.2	<2 / <1	<2	2.3	5.4	0.96
16	5.3	<2 / <1	<2	<1	5.1	0.95
17	6.1	<2 / <1	<2	3.4	5.6	0.93
18	6.4	<2 / <1	7.6	<1	5.3	0.95
19	7.3	<2 / <1	3.7	3.7	5.5	0.95
20	7.9	<2 / <1	8.0	3.1	5.6	0.96
21	8.1	<2 / <1	5.8	<1	5.6	0.95
22	8.2	3.0 / 3.3	3.9	3.0	5.4	0.95
23	8.2	2.6 / <1	7.5	<1	5.8	0.94
24	8.9	2.0 / 1.5	8.0	3.0	5.2	0.97
25	6.6	<2 / <1	3.8	<1	5.5	0.92

*: coagulase-negative colonies

Relation of biogenic amine contents with bacterial numbers, pH and water activity

Formation of biogenic amines by bacteria is a widely studied phenomenon. In particular, numerous Enterobacteriaceae are able to form putrescine and cadaverine, and sometimes also histamine, from the precursor amino acids (Özogul and Özogul, 2007). This reaction is often an attempt to counterbalance acidic or other stressful environments (Beutling, 1996). Tyramine formation is more frequently observed among enterococci and lactic acid bacteria (Pircher et al., 2007), and, in particular, PHE formation by enterococci has been studied by Beutling and Walter (2002). Notably, this compound demonstrated antibacterial activity in meat surrogates and in beef (Lynnes et al., 2014), but its potential effect on the microflora of cheese remains to be investigated. Polyamines, as spermidine and spermine, are, in contrast, the result of synthesis processes associated with cell growth and thus, an increase of polyamine contents (per fresh matter) during cheese production is likely attributable to drying (Novella-Rodríguez et al., 2003). Since cheese manufacture is a complex process, contents of biogenic amines could originate from the activity of bacteria present in the milk before curd production, or from microbiota originating from the dairy equipment. In addition, the amine-forming capability within a species is rather strain-specific (Özogul and Özogul, 2007). It was thus not unexpected that no significant relation could be established between the contents of single amines or the sum of all amines to log numbers of bacterial groups under study. There was, however, a significant (P≤0.05) positive correlation of pH with contents of PHE (r=0.44), cadaverine (r=0.44) and putrescine (p=0.37) on one side, and a negative correlation of water activity and PHE (–0.56), cadaverine (–0.53) and histamine (–0.47) on the other side. Since the correlation coefficients are only moderately strong to weak, these associations should be interpreted with caution. Whereas the finding that higher pH would correlate with higher contents of some amines was not expected, higher amine contents and lower water activity could be related, e. g. via drying, salt content (inducing osmotic stress), proteolysis (which would provide precursor amino acids and increase osmotic pressure). It was not in the scope of this study to evaluate technological and physicochemical properties of cheeses in terms of their ability to induce bacteria amine formation, but further tests will consider this issue.

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Conflict of interest

The authors declare that they have no conflict of interest.

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