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Biogenic Amine Content in Retailed Cheese Varieties Produced with Commercial Bacterial or Mold Cultures

Nevijo Zdolec [1], Tanja Bogdanović [2], Krešimir Severin [3], Vesna Dobranić [1], Snježana Kazazić [4], Jozo Grbavac [5], Jelka Pleadin [6], Sandra Petričević [2] and Marta Kiš [1,*]

1 Department of Hygiene, Technology and Food Safety, Faculty of Veterinary Medicine, University of Zagreb, 10 000 Zagreb, Croatia; nzdolec@vef.hr (N.Z.); vdobranic@vef.hr (V.D.)
2 Veterinary Center Split, Croatian Veterinary Institute, 21 000 Split, Croatia; t.bogdanovic.vzs@veinst.hr (T.B.); petricevic.vzs@veinst.hr (S.P.)
3 Department of Forensic and State Veterinary Medicine, Faculty of Veterinary Medicine, University of Zagreb, 10 000 Zagreb, Croatia; severin@vef.hr
4 Division of Physical Chemistry, Ruđer Bošković Institute, 10 000 Zagreb, Croatia; Snjezana.Kazazic@irb.hr
5 Faculty of Agriculture and Food Technology, University of Mostar, 88 000 Mostar, Bosnia and Herzegovina; grbavac.jozo@gmail.com
6 Laboratory for Analytical Chemistry, Croatian Veterinary Institute, 10 000 Zagreb, Croatia; pleadin@veinst.hr
* Correspondence: mkis@vef.hr; Tel.: +385-1-2390-199

Abstract: Biogenic amines (BAs) are considered a potential microbiological toxicological hazard in aged cheese. Risk mitigation strategies include good hygiene practice measures, thermal treatment of milk and the use of competitive dairy cultures. The aim of this study was to evaluate the amount of BAs—tryptamine, β-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine—in the core and rind of cheeses ripened by bacteria (n = 61) and by mold cultures (n = 8). The microbial communities were counted, and the dominant lactic acid bacteria (LAB) were identified, corresponding to the BA concentrations. The total BA content was highest in the core of semi-hard cheeses (353.98 mg/kg), followed by mold cheeses (248.99 mg/kg) and lowest in hard cheeses (157.38 mg/kg). The highest number of BAs was present in the rind of cheeses with mold (240.52 mg/kg), followed by semi-hard (174.99 mg/kg) and hard cheeses (107.21 mg/kg). Tyramine was the most abundant BA, represented by 75.4% in mold cheeses, 41.3% in hard cheese and 35% of total BAs in semi-hard cheeses. Histamine was present above the defined European maximum level (ML) of 100 mg/kg in only two semi-hard and three hard cheeses. High amount of BAs (above 600 mg/kg) in cheeses, mainly tyramine, were associated with the presence of *Enterococcus durans*, while negligible BA concentrations were found in cheeses ripened with *Lacticaseibacillus rhamnosus*, *Lactococcus lactis* or *Lacticaseibacillus paracasei* cultures. This study has shown that retailed cheese varieties produced with commercial bacterial or mold cultures have acceptable levels of biogenic amines with respect to consumers.

Keywords: biogenic amines; enterococci; lactobacilli; lactococci; ripened cheese

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1. Introduction

Biogenic amines (BAs) are low molecular weight compounds associated with the decarboxylation activity of microorganisms in fermented foods, particularly in aged cheeses [1,2]. Although they have a number of different regulatory functions in animal and plant tissues, as well as in microbial cells, their formation and sustainability in food requires attention, as consumption of foods containing large amounts of BAs may have serious toxicological consequences [2]. They are formed during the ripening and storage of cheese, and the factors affecting BA formation are pH, salt concentration, bacterial activity, humidity, storage temperature and ripening time [3]. In ripened cheeses, the BAs detected in the highest concentrations are tyramine, cadaverine, histamine and putrescine [2,4]. The total numbers of BAs in different cheeses available in the market vary considerably, reaching

values up to 257.71 mg/kg for hard cheese and 384.33 mg/kg for semi-hard cheese in Korea [5], 407.8 mg/kg in hard and semi-hard cheeses and up to 327.5 mg/kg in mold-ripened cheese in Croatia [6] and 1875.43 mg/kg in hard cheese in Egypt [7]. However, BA concentrations can further increase during cheese storage, i.e., during shelf life. In a recent study, Dabadé et al. [8] reported a tyramine concentration of 1029 mg/kg in a semi-hard cheese at its expiry date. Hard cheeses were found to have stable and high concentrations of tyramine and histamine (1025 mg/kg) during storage, while most of the amines were found to be tyramine and cadaverine (1306 mg/kg) in blue cheeses. The average concentrations of BAs in the samples of hard cheeses (e.g., Parmesan) were found to be significantly higher than in other cheeses.

Some of these high amounts have toxicological significance for consumers when compared with the set European maximum level (MLs) of histamine in fish of 100 mg/kg [9]. Indeed, for other foods, especially fermented foods, there are no regulated set MLs for individual or total amines. Kandasamy et al. [5] reported that although the tolerance limit for toxic amines in cheese is 200 mg/kg, amine amounts of 1889.75 mg/kg and 1237.80 mg/kg were found in extra hard cheeses (imported Pecorino Romano and Grana Padano). Ladero et al. [10] consider the difficulty in establishing MLs for individual amines in foods, given insufficient toxicological studies; since different BAs are simultaneously present in foods, the MLs of 750–900 mg/kg can be recommended. On the other hand, Ladero et al. [10] emphasize that it would be reasonable to set preventive "m"/"M" limits of 200 and 500 mg of individual BA per kg for foods that are likely to contain many BAs, such as cheese.

Given the aminogenic potential of microorganisms, it is crucial to know the composition of the cheese microbiota and the possible relationship between microbial activity and the formation of amines in them. The microbiological properties of cheese depend on the type of cheese, i.e., hygiene and production technology, especially heat treatment of milk and the use of dairy cultures [4,11]. The microbiota of raw milk under poor hygiene conditions may contain pathogenic bacteria, such as *Listeria monocytogenes* and *Staphylococcus aureus*, but also fecal or environmental contaminating bacteria, such as coliforms or enterococci [12–14]. In this context, coliform bacteria and lactic acid bacteria (LAB), mainly enterococci, are responsible for the formation of biogenic amines in unprocessed dairy products [15,16]. In general, higher microbiological contamination, i.e., a higher total number of bacteria in raw material, may also cause a higher formation of BAs in cheese made from unpasteurized milk [7].

However, correlations between microbiological counts and end-of-storage concentrations of BAs (or trends in BA concentrations) have generally been weak for various commercially available foods, including cheese [8]. This can be explained by the low sensitivity of culturable microbiological methods and the reduction of microbial populations during extended cheese storage. For example, Vrdoljak et al. [17] reported a reduction of LAB in hard cheeses from 7 logs to 2 logs during 450 days of storage as well as from 6.2 to 4.6 logs in semi-hard cheeses during a 270-day period. In addition to the total number of microorganisms, such as aerobic mesophiles, psychrotrophs, enterobacteria or LAB, involved in the production of BAs, the presence of certain species may be important in evaluating the accumulation or even reduction of BAs in cheese. The microbial species most commonly associated with the formation of the major BAs found in food are *Enterococcus faecalis*, *E. faecium* and *E. durans* (tyramine), while others, such as *Lactiplantibacillus plantarum*, *Latilactobacillus sakei*, *Lactiplantibacillus pentosus* and *Pediococcus acidilactici*, degrade tyramine and histamine [18,19].

One of the strategies to reduce the formation of BAs in ripening cheeses is to make use of competitive dairy cultures due to their possible inhibitory effect on amine-producing bacteria.

Therefore, the objective of this study was to select retail semi-hard and hard cheeses produced with the addition of bacterial cultures to gain insight into the amounts of BAs and their possible connection with microbial populations. The study assumed that cheeses

incorporated with bacterial dairy cultures would contain low amounts or at least toxicologically acceptable amounts of the main BAs. In addition, the quantities of BAs in commercially available soft cheeses aged with selected mold cultures were evaluated.

2. Materials and Methods

2.1. Cheese Samples

Samples of local and imported cheeses (n = 69) in the original retail packaging were collected for the study (Table 1). All cheeses were made from pasteurized cow, goat or sheep milk and ripened by mold or bacterial cultures. The mold-ripened cheese group consisted of surface-ripened white mold cheeses (Brie/Camembert type, n = 3) and internal mold-ripened, blue-veined cheeses (Gorgonzola type, n = 5). The group of semi-hard cheeses (n = 18) consisted of internal-bacterial ripened varieties (Trappist, Dutch type) from 6 producers. The group of hard cheeses (n = 43) consisted of the cheese type Parmigiano-Reggiano, internal-bacterial ripened cheeses with or without additional ingredients (fruits and wine), and came from 9 producers.

Table 1. Characteristics of retail cheese selected in this study.

Cheese Group, Number of Samples	Properties (Microbial Cultures Involved in Ripening)	Milk
Mold-ripened (n = 8)	Surface-ripened—white mold cheese—Brie/Camembert type (Germany, n = 3)	Cow
	Internal mold—blue-veined—Gorgonzola type (Croatia, Italy, Netherlands, n = 5)	Cow (n = 4) Goat (n = 1)
Semi-hard cheese (n = 18)	Internal-bacterial ripened—Trappist type (Croatia, * n = 5, ** n = 1; Bosnia and Herzegovina, *** n = 5); Internal-bacterial ripened, rennet (Bosnia and Herzegovina, **** n = 5) Dutch type cheese (New Zealand, ***** n = 2)	Cow
Hard cheese (n = 43)	Parmigiano-Reggiano cheese type (Croatia, * n = 5), Internal-bacterial ripened (Bosnia and Herzegovina, ** n = 5, Croatia, *** n = 10), Internal-bacterial ripened (Croatia, **** n = 15)	Cow Cow (n = 5), goat (n = 5), ewe (n = 5)
	Internal-bacterial ripened (Croatia, ***** n = 3)	Ewe
	(Croatia, ****** n = 1)	Ewe
	(Croatia, ******* n = 1),	Cow
	Internal-bacterial ripened with wine (Croatia, ******** n = 1),	Cow
	Internal-bacterial ripened with apricot/cranberry (Great Britain, ********* n = 2)	Cow

* Different asterisks represent different producers within cheese group.

2.2. Microbiological Analyses

For microbiological analysis, 25 g of cheese was taken after sterile separation of the surface layers of the product. The test sample was diluted in 225 mL of buffered peptone water (Biolife, Milano, Italy) and homogenized at 200 rpm for 2 min (Stomacher, Seward, UK). Serial decimal dilutions were then prepared to determine the number of aerobic mesophilic bacteria, LAB, enterococci, yeasts and molds, enterobacteria and *Escherichia coli*. The number of aerobic mesophilic bacteria was determined on plate count agar (PCA, bioMerieux, Craponne, France) by incubation at 30 °C for 72 h. The number of LAB was determined on de Man–Ragosa–Sharpe agar (MRS, Merck, Darmstadt, Germany) and incubated at 30 °C for 24–48 h, and enterococci on Compass Enterococcus agar chromogenic medium (BIOKAR, Beauvais, France) by incubation at 37 °C for 24 h. The number of yeasts and molds was determined on oxytetracycline glucose yeast agar (Merck, Darmstadt, Germany) by incubation at 25 °C for 48–72 h. The number of *E. coli* was determined on

rapid *E. coli* chromogenic medium (BIOKAR, Beauvais, France) and incubated at 37 °C for 24 h, and enterobacteria on violet red bile agar with glucose (VRBG, Merck, Darmstadt, Germany) by incubation at 37 °C for 24 h. Before counting, catalase, oxidase and coagulase tests were performed for LAB, enterobacteria and staphylococci, respectively. The results are expressed as logarithmic values of the numbers of colonies per gram of cheese ($\log_{10}$ CFU/g).

2.3. Identification of Dominant Lactic Acid Bacteria (LAB)

Colonies grown on MRS agar were selected from the highest sample dilutions of semi-hard and hard cheeses and determined to the species level by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonik, Bremen, Germany). A sample for MALDI TOF MS analysis was prepared following the extended direct transfer procedure recommended by the manufacturer (Bruker Daltonik, Bremen, Germany). A single colony was smeared onto a 96-spot polished steel target plate, applied with 1 µL of 70% formic acid (Fisher Scientific, Madrid, Spain) and dried at room temperature. Each sample was overlaid with 1 µL of MALDI matrix (a saturated solution of α-cyano-4-hydroxycinnamic acid, HCCA, Bruker Daltonik, Bremen, Germany) in 50% acetonitrile and 2.5% trifluoroacetic acid (Sigma-Aldrich, St. Louis, MO, USA) and air dried at room temperature. Mass spectra were automatically generated using a microflex LT MALDI TOF mass spectrometer (Bruker Daltonik, Bremen, Germany) operated in the linear positive mode within a mass range of 2000–20,000 Da. The instrument was calibrated using a Bruker bacterial test standard. Recorded mass spectra were processed with the MALDI Biotyper 3.0 software package (Bruker Daltonik, Bremen, Germany), using standard settings. The MALDI Biotyper output is a log score value in the range 0–3.0, representing the probability of correct identification of the isolate, computed by comparison of the peak list for an unknown isolate with the reference spectrum in the database. The identification criteria used were as follows: a score of 2300–3000 indicated highly probable species level identification, a score of 2000–2299 indicated secure genus identification with probable species identification, a score of 1700–1999 indicated probable identification to the genus level and a score of <1700 was considered unreliable.

2.4. Determination of Biogenic Amines (BA)

The 8 biogenic amines studied (cadaverine—CAD, histamine—HIS, phenylethylamine PHE, putrescine—PUT, spermidine—SPD, spermine—SPM, tryptamine—TRP and tyramine—TYR) were detected and quantified by high performance liquid chromatography, using a diode array detector (G1315B DAD, Agilent Technologies, Santa Clara, CA, USA) at 254 nm, with 550 nm as a reference after precolumn derivatization with dansyl chloride, as described by Eerola et al. [20] and Bogdanović et al. [6]. The extraction of a 5 g homogenized cheese sample (from both the cheese core and cheese rind) was performed in 50 mL 0.4 mol/L perchloric acid. A total of 250 µL of an internal standard solution (1,7 heptanediamine, 1000 mg/L) was added to the homogenized sample prior to the extraction with 0.4 M perchloric acid. One mililiter of perchloric cheese extract was derivatized according to Bogdanović et al. [6]. Each food sample was analyzed in triplicate, the BA content stated herein represents the mean of these three parallel analyses. BA concentrations are expressed as mg/kg (wet weight).

Chromatographic separation of 8 BAs of interest was performed by high-performance liquid chromatography in combination with a diode array detector (HPLC-DAD, Agilent Technologies 1200 Series HPLC, Santa Clara, CA, USA), according to Bogdanović et al. [6]. The precise instrument module details and HPLC-DAD parameters were as described in Supplementary Materials. The compounds were quantified using internal calibration curves plotted for each BA and covering 8 concentration levels ranging from 0.25–500 mg/kg. The method used for biogenic amine determination in cheese was validated according to the criteria laid down in the EC Regulation No. 333/2007. The performance assessment criteria included the applicability, limit of detection (LOD), limit of

quantification (LOQ), precision (RSDr), specificity, linearity and recovery. The precision of the method was assessed at three concentration levels under repeatable conditions (RSDr). Cheese samples were spiked at the concentrations of 50, 100 and 250 mg/kg in triplicate. The presence or absence of matrix effects was identified using calibration curves and a fixed amount of internal 1,7 heptane diamine (HEP) standard (50 mg/kg) obtained with matrix-matched calibration standards and calibration solutions in the solvent. Internal quality control was pursued with each analytical batch using the available quality control material (canned fish T27137QC, HIS assigned value: 212 ± 30 mg/kg, Fapas, York, England), and was carried out by virtue of spiking the cheese samples so as to obtain the concentration of 100 mg/kg. Within each analytical series, the reference materials and the spiked cheese samples were analyzed in duplicate and checked for recovery. The applied analytical method fulfils all methodological requirements set out under the EC Regulation No. 333/2007 and can therefore be considered as suitable for the determination of 8 BAs in food groups under this study. The results concerning linearity, LOD, LOQ, recovery and RSDr are presented in Supplementary Materials.

2.5. Statistical Analysis

Statistical analysis of the results was performed using standard descriptive statistical methods (Statistica 13.5, TIBCO Software, Palo Alto, Santa Clara, CA, USA) by determining the arithmetic mean (x) with the standard deviation (SD) and the minimum (min) and maximum (max) values. Kruskal–Wallis analysis of variance at the 0.05 probability level was used to determine statistically significant differences between the cheeses, and post-hoc analysis was used to determine the differences between each group as well as between different producers within the same cheese group. The correlation between the individual values of BAs and bacteria was determined with the Spearman correlation coefficient (r_s).

3. Results and Discussion

Results of microbiological analyses of soft cheeses with mold, semi-hard and hard cheeses are presented in Table 2.

Table 2. Microbial population counts ($\log_{10}$ CFU/g) in different cheese types.

Microorganisms	Mold-Ripened (n = 8)		Semi-Hard Cheese (n = 18)		Hard Cheese (n = 43)	
	Mean ± SD	Min/Max	Mean ± SD	Min/Max	Mean ± SD	Min/Max
Aerobic mesophilic bacteria	5.74 ± 1.42 ^{a}	4.04–7.77	7.30 ± 0.46 ^{ab}	6.25–7.95	5.74 ± 1.80 ^{b}	2.00–7.80
Enterococci	4.18 ± 1.21	2.69–5.90	5.03 ± 2.20	2.00–8.55	5.20 ± 1.14	2.30–6.60
Lactic acid bacteria	6.84 ± 1.09	5.00–7.90	7.57 ± 0.97 ^{a}	4.00–8.50	6.60 ± 0.93 ^{a}	4.69–8.00
Yeasts and molds	6.49 ± 1.30 ^{ab}	5.00–7.69	3.43 ± 1.48 ^{a}	2.00–7.95	3.10 ± 1.07 ^{b}	2.00–6.00
Enterobacteriaceae	2.38 ± 0.49	1.69–3.00	3.06 ± 0.56	2.00–3.50	2.23 ± 0.91	1.00–3.80
Staphylococci	3.73 ± 0.65	2.69–4.47	3.82 ± 0.98	2.69–6.30	3.80 ± 0.34	2.84–4.38
Escherichia coli	<2.00	<2.00	<2.00	<2.00	3.81 ± 0.23	3.50–4.07

Values in the same row marked with the same letter denote statistically significant differences ($p < 0.05$).

Table 2 shows that LABs are the dominant microbiota in all investigated cheese types, with an average number of 6–7 $\log_{10}$ CFU/g. A stable population of enterococci (members of the LAB group) in the number of 4–5 $\log_{10}$ CFU/g and an equal number of coagulase-negative staphylococci (3.5 $\log_{10}$ CFU/g) is also observed. The number of yeasts and molds is almost the same in semi-hard and hard cheeses, and significantly different ($p < 0.05$) from their number in soft cheeses (6.49 $\log_{10}$ CFU/g) due to the technological process involving molds. *Enterobacteriaceae* were found occasionally in all cheese groups, but the result was mainly related to individual producers. Surprisingly, *E. coli* was also present in hard cheeses (n = 5) from one producer. A wide range of results can be observed in all studied microbial populations grouped by cheese types (soft cheese with mold, semi-hard cheese, and hard cheese), indicating significant differences in production technology, dairy culture activity or hygienic production conditions. Microbiological changes in/on cheese

during production or storage depend on various factors, such as production technology and type of cheese (pasteurization of milk or raw milk, use of dairy cultures, acidity and ripening), physicochemical properties of cheese and storage conditions [21]. Lactic acid bacteria are technologically the most important microorganisms in cheese production, and their numbers in the studied cheeses depends on the type of product, i.e., conditioned application of dairy cultures. Further development of LABs in cheese during storage depends on the type of cheese, consequently it generally decreases in semi-hard and hard cheeses and increases in soft cheeses [17]. As far as contaminating microorganisms are concerned, the findings of enterobacteria, *E. coli* and enterococci may indicate poor quality of the raw material used and deficiencies in the milk pasteurization. Bacterial thermoresistance is well documented, especially in enterococci, thus lower temperatures applied in cheese technology may not affect their population, as was the case for the amine-producing *Enterococcus durans* in the study by Ladero et al. [22]. Moreover, the possibility of the contamination of the cheese after processing should not be ignored. Enterococci are known to be controversial ubiquitous bacteria, and their presence in cheese is not necessarily an indicator of fecal contamination [23]. In any case, these microorganisms have a high aminogenic potential and can influence the content of biogenic amines, especially tyramine in cheese [24]. Coagulase-negative staphylococci were found in equal numbers in all cheese types and, together with LAB, are technologically/safety important dairy bacteria, but health risks can be expected if resistant and amine- or enterotoxin-producing strains are present [25,26].

Table 3 shows the determined concentration of each BA analyzed in the core and rind of soft cheeses with mold, semi-hard cheeses and hard cheeses. The total content of amines in the core was highest in semi-hard cheese (353.98 mg/kg), followed by mold cheese (248.99 mg/kg) and lowest in hard cheese (157.38 mg/kg). Among the surface samples, the highest content of BA was present in cheese with mold (240.52 mg/kg), followed by semi-hard (174.99 mg/kg) and hard cheese (107.21 mg/kg). In contrast to soft and hard cheeses, in the semi-hard cheese group, the amounts of all amines were higher in the middle than in the rind, and statistically significant differences were found for putrescine, tyramine and spermine ($p < 0.05$). Considering the amounts of BAs in the core and rind of the cheese, a positive correlation was found in all three cheese groups: soft cheese (r = 0.97), semi-hard cheese (r = 0.78) and hard cheese (r = 0.91). It is well known that BA content varies between different aged cheeses, within the same cheese type and within cheese parts [27]. This study shows that BA content is lowest in hard, long-ripened cheeses, which contrasts with other findings claiming a direct effect of ripening time and intense proteolytic changes with accumulation of BAs [7,27,28]. The obtained differences between mentioned studies may be explained by the competitiveness of lactic starters against aminogenic non-starter lactic acid bacteria (NSLAB). For example, the lowest BA content represented only by cadaverine (<0.61–1.28 mg/kg) and spermidine (<0.39–2.57 mg/kg) was found in a Croatian variety of Parmigiano-Reggiano cheese aged for 1–3 years, and the dominant isolated culture was *Lacticaseibacillus rhamnosus*, without enterococci. Shalabi et al. [29]. demonstrated high anti-tyramine potential of *Lacticaseibacillus rhamnosus* against tyrosine decarboxylase gene carrying strains isolated from cheese. In general, the total content of BA in cheese ripened by bacteria or molds in this study is 2–5 times lower than the toxicological limits proposed by Ladero et al. [10].

Table 3. Biogenic amine content in retailed cheese varieties ripened with mold or bacterial dairy cultures.

	Mold-Ripened (*n* = 8)				Semi-Hard Cheese (*n* = 18)				Hard Cheese (*n* = 43)			
BAs	**Core**		**Rind**		**Core**		**Rind**		**Core**		**Rind**	
	Mean	**Min–Max**	**Mean**	**Min–Max**	**Mean**	**Min–Max**	**Mean**	**Min–Max**	**Mean**	**Min–Max**	**Mean**	**Min–Max**
TRP	<0.77	<0.77	<0.77	<0.77	<0.77	<0.77	<0.77	<0.77	9.01 ^{a}	1.56–23.89	5.58 ^{a}	2.55–19.62
β-PHE	42.90	1.43–84.38	30.03	<0.63–84.18	46.55	1.07–130.59	18.81	1.39–55.25	16.05	0.64–52.44	24.55	10.08–92.85
PUT	6.38	0.64–30.53	4.39	1.01–8.04	15.93 ^{Aa}	1.37–95.21	9.78 ^{Ba}	0.80–62.56	3.31 ^{A}	<0.59–11.63	2.28 ^{B}	<0.59–9.13
CAD	2.57 ^{A}	<0.61–5.38	3.70 ^{B}	2.35–4.70	64.22 ^{A}	0.76–436.68	38.17 ^{B}	<0.61–292.80	19.48 ^{A}	<0.61–119.38	12.77	<0.61–83.85
HIS	7.05	0.74–13.99	6.23	2.59–13.07	87.82	13.5–248.55	40.83	4.17–127.01	28.35	<0.59–116.42	20.96	<0.59–85.90
TYR	183.97	0.97–710.5	185.13	1.86–62.75	129.96 ^{Aa}	2.30–767.03	55.13 ^{a}	<0.89–376.66	72.60 ^{A}	1.33–236.33	36.69	<0.89–142.81
SPD	6.12	1.75–16.58	11.04	1.62–22.24	7.03 ^{A}	3.35–17.90	11.65	0.50–66.53	5.35 ^{A}	<0.39–21.70	4.38	<0.39–18.17
SPM	<1.01	<1.01	7.32	<1.01–15.64	2.47 ^{a}	<1.01–9.02	<1.01 ^{a}	<1.01–1.15	3.23	<1.01–5.12	10.05	<1.01–10.96

n—number of samples, BAs—biogenic amines, TRP—tryptamine, β-PHE—β-phenylethylamine, PUT—putrescine, CAD—cadaverine, HIS—histamine, TYR—tyramine, SPD—spermidine, SPM—spermine. Values expressed as < (less than) denoted values lower than the method detection limit. Uppercase letters (A or B) in the same row denote statistically significant differences ($p < 0.05$) between cheese groups (mold-ripened, semi-hard, hard cheese); a lowercase letter (a) in the same row denotes statistically significant differences ($p < 0.05$) between core and rind within the cheese group.

Regarding the percentage of each BA in total BAs, tyramine was the most abundant amine, accounting for 75.4% in mold cheese, 41.3% in hard cheeses, and 35% of the total amines in semi-hard cheeses. These results are consistent with other studies showing that tyramine is the dominant BA in various cheeses, including those investigated in this study [6,29]. The average tyramine content was below 200 mg/kg; however, there were large differences between individual samples and even within the same cheese varieties, as previously reported [6]. Among individual samples, a mold-ripened cheese and a semi-hard cheese had the highest tyramine concentrations of 762.75 mg/kg and 767.03 mg/kg, respectively.

The other BAs were present across a wide spectrum in all the cheeses studied, except for tryptamine, which was found in low concentrations only in hard cheeses. The second most abundant amine in semi-hard and hard cheeses was histamine, and its content was higher in the core than in the rind in both cheeses, which was found by Marijan et al. [1] in hard cheeses, but without statistical significance in case of this study ($p > 0.05$). Although no statistically significant differences were found in histamine content between cheeses, significant differences were observed within the group of semi-hard cheeses between the producers of these cheeses ($p < 0.05$). Only two semi-hard cheeses and three hard cheeses had histamine amounts above the 100 mg/kg; defined in the European Union as ML level [30]. Histamine is the most toxicologically significant of all amines and frequently exceeds maximum food safety levels, and its concentrations are reported to be higher in raw milk cheeses than in processed cheeses [27]. The greatest differences between cheese types were found for cadaverine, whose amounts were 2.57–64.22 mg/kg in the core of the cheese and 3.70–38.17 mg/kg in the rind ($p < 0.05$). The high variability of cadaverine concentrations in cheese was also reported by Bunka et al. [31]; however, maximum values determined in this study were several times lower.

Comparing the association of the BAs found with the isolated microbiota, numerous differences were found according to the cheese group and within the producer. In the semi-hard cheese group, the number of enterococci and staphylococci was found to be statistically significantly positively correlated (r > 0.6) with the amount of ß-phenylethylamine, histamine and tyramine, but differed between producers. LAB as well as yeasts and molds reduce the synthesis of certain BAs, which has been confirmed by a negative correlation

factor ($r > -0.49$) in the case of histamine, putrescine and tyramine. The amine-reducing ability of LABs, such as lactobacilli or lactococci, has been previously reported in aged cheese [32,33]. In this study, *Lacticaseibacillus paracasei* and *Lactococcus lactis* were isolated from LAB populations (highest dilutions) of semi-hard and hard cheeses, which had a very low total amount of BAs. It can be assumed that their dominance in the cheese microbiota resulted in lower BA content in the cheeses from this study.

In the group of semi-hard cheeses, the number of *Enterobacteriaceae* correlated positively with the value of putrescine ($r = 0.81$) and negatively affected spermin synthesis ($r = -0.76$). In hard cheeses, their number correlated with the value of ß-phenylethylamine, histamine and spermine. The aminogenic potential of enterobacteria is well known, especially in the formation of putrescine, and their presence is the consequence of poor hygienic practices in cheese production [10]. Although enterococci are associated with a risk of biogenic amines, their differential effect on BA synthesis has been observed. For example, at a concentration of 4.5 $\log_{10}$ CFU/g, their number was significantly positively correlated with the amount of spermidine ($r = 0.9$) in semi-hard cheeses from the same producer, while a negative correlation was observed in another cheese at a concentration of 8 $\log_{10}$ CFU/g ($r = -0.94$). Such discrepancies may be due to different production conditions, as inappropriate environmental factors, such as pH, temperature or salt concentration, may influence BA production [34]. The most common species of enterococci in milk are *Enterococcus faecium*, *Enterococcus durans* and *Enterococcus faecalis*, most of which have been identified as tyramine and putrescine producers [10,35]. In this study, in two varieties of semi-hard and hard cheeses, the dominant species within LAB was *E. durans*, and these cheeses had the highest BA values, of which tyramine was found at the highest concentrations. Considering that enterococci are usually not present in the function of starter cultures and that their introduction is the result of subsequent contamination (NS-LAB), it is expected that this is the case with analysis of these types of cheeses, which confirm the presence of high numbers of enterobacteria and staphylococci in them.

4. Conclusions

This study has shown that retailed cheese varieties produced with commercial bacterial or mold cultures have acceptable levels of biogenic amines with respect to consumers. The microbial communities examined were generally associated with the accumulation or reduction of BAs in the cheese samples, firstly, by the dominance of enterococci and, secondly, by the dominance of lactobacilli or lactococci. This was evident in individual producers of semi-hard cheeses, where the largest populations of enterococci and staphylococci correlated with the highest concentrations of BAs, especially tyramine, histamine, cadaverine, putrescine and β-phenyletilamine. Unlike enterococci, the presence of LAB is likely to be associated with a reduction in the synthesis of BAs, but, given the many variations in their counts, to confirm this it will be necessary to evaluate their activity patterns in the controlled production of different cheeses. According to the obtained results, the aminogenic or amine-reducing capacity of the bacterial strains collected from the cheeses in this study will be further investigated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10010010/s1, Reagents and materials for biogenic amines determination and quantification; HPLC-DAD analysis; Table S1: Selected performance indicators of the method in use: linearity, limit of detection (LOD), limit of quantification (LOQ), recovery and precision (RSD_r).

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