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Biogenic amines occurrence in beers produced in Czech microbreweries

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Abstract

The objective of the current study was the evaluation of 8 biogenic amines (BA) occurrence in beer samples (115 samples in total) manufactured in microbreweries of the Central Europe region in relation to the progress of the storage period (at 20 ± 2 °C). The examined beer samples were divided into 3 main groups according to their extract of original wort value (**EOW**): (i) Light craft beer (EOW ≤ 10; 12 samples in total), (ii) Lager craft beer (11 ≤ EOW ≤ 12; 65 samples in total), (iii) Special craft beer (EOW ≤ 13; 38 samples in total). The tested craft beer samples were analyzed immediately after purchase and at the end of the best before date. Furthermore, the most frequently detected BA was tyramine. In addition, other abundant monitored BA were putrescine and cadaverine. Moreover, concentrations of histamine above 20 mg/l were detected in lager craft beer and special craft beer samples (at the end of the best before date). On the whole, with the progress of the storage time the BA concentration increased. Thereafter, more than 30% of the tested samples presented total BA content in the range of 50 – 100 mg/l. However, 18% of the examined craft beer samples had a total amount of BA higher than 100 mg/l.

Key words: beer; biogenic amine; microbreweries; hazard;

1 Introduction

Biogenic amines (**BA**) are the group of low-molecular nitrogen compounds derived from amino acids and are developed in foods mainly by the action of decarboxylases, but also by transamination or amination of aldehydes and ketones (Buňková et al., 2013; Halász, Baráth & Holzapfel, 1999; Shalaby, 1996; Silla Santos, 1996). Moreover, according to the chemical structure BA could be categorized into the following groups: (i) aliphatic – putrescine (**PUT**), cadaverine (**CAD**), spermine (**SPN**), spermidine (**SPD**), agmatine (**AGM**); (ii) aromatic – tyramine (**TYM**), phenylalanine (**PHE**); and (iii) heterocyclic – histamine (**HIM**), tryptamine (**TRM**). Furthermore, BAs are indispensable compounds during some physiological processes and organisms can synthesize BAs themselves. Hence, upon food and beverage intake, the human body is able to metabolize BAs, primarily through the action of monoamine oxidases, diaminooxidases and histidine-methyltransferate (Loret, Deloyer & Dandrifosse, 2005; Tofalo, Perpetuini, Schirone & Suzzi, 2016). However, with a high BA intake, the detoxification mechanism may be insufficiently effective and the health of the consumer may be endangered and, in extreme cases, this may lead to death. In general, concentrations of BAs up to 100 mg/kg or 100 mg/l are considered to be safe for the consumer. Nevertheless, various compounds such as ethanol and various drugs can significantly reduce the effectiveness of the detoxification mechanism (Fusek, Michálek, Buňková, & Buňka, 2020; Halasz et al., 1994; Shalaby, 1996). Therefore, the recommended limits for alcoholic beverages are much lower and, for some certain BAs, may be units of milligrams per kilogram or liter (Tofalo et al., 2016). Moreover, BAs can be detected in (i) fermented foods where they are produced by the applied starter cultures; but also in (ii) non-fermented foods where they may be formed by the presence contaminating microorganisms. In the case of fermented foods, contaminating microflora might also be present and might be capable of BA formation.

Beer is a carbonated alcoholic beverage widely consumed around the world, being the first among the alcoholic beverages (Adamenko, Kawa-Rygielska, & Kucharska, 2020; Lorencová, Salek, Černošková, & Buňka, 2019). In general, beer is regarded to be a safe product in terms of foodborne illnesses due to its remarkable microbiological stability. Thus, pathogens and other microorganisms are not able to grow in the "environment" of beer because of the presence of ethanol and hop bitter compounds, high contents of CO_2, low pH and reduced O_2 level. However, bacterial contamination by spoilage microorganisms is possible (Maifreni et al., 2015; Sakamoto & Konings, 2003). Thus, beer could be characterized as a BA formation favorable "environment" (beverage) (Lorencová et al., 2012). The most common sources of BA in beer are the applied raw materials (especially malt) and subsequently, the aforementioned contaminating microflora [mainly lactic acid bacteria (**LAB**)]. Thereafter, important contaminants from among the group of LAB are representatives of the genera *Lactobacillus*, has a significant effect on the levels of BA, in particular for beers with prolonged storage time (Kalač, Hlavatá & Křížek, 1997; Kalač, Šavel, Křížek, Pelikánová & Prokopová, 2002; Lorencová et al., 2012; Poveda, Ruiz, Sesena & Palop 2017; Suzuki, Asano, Iijima, Kitamoto, 2008).

Monitoring of BA content in beers is carried out worldwide. In particular, it is worthy to mention the studies of Deetae, Perello & de Revel (2013), Izquierdo-Pulido, Hernánder-Jover, Mariné-Font & Vidal-Carou (1996a; 1996b), Loret et al. (2005), Slomkowska & Ambroziak (2002) delineating with beer samples from Spain, Belgium, France and Poland, respectively. The above-mentioned authors stated that beer produced on an industrial scale generally comprise of small amounts of milligrams per liter (or kg) of BA. However, in some cases the detected concentrations of BA were above 50 mg/l or even above 100 mg/l. Moreover, the content of BA in beers produced under industrial breweries of the Central Europe region was previously reported by Buňka et al. (2012) and Kalač et al. (2002). The latter authors stated that beer samples from the region of the Central Europe can contain on the order of units to a few tens of milligrams of BA per liter of beer. Additionally, Czech Republic is the country with the highest rate of beer consumption per capita (144 L) followed by Austria and Germany (both 108 L per capita) (Pradenas, Galarce-Bustos, Henriquez-Aedo, Mundaca-Uribe & Aranda., 2016). In addition, microbrewing has become a well-established segment of the brewing industry in Europe, North America, Asia and Oceania. Hence, the Czech Republic a country of the Central European region, in which the production and consumption of beers produced in microbreweries (which are generally considered to be producers with an annual beer production lower than 10,000 hl) became a driving force in order to introduce or to develop many divergent types of beer with varying characteristics. The production conditions in microbreweries are usually significantly different from industrial plants with advanced automation and mechanization systems. In addition, beers from microbreweries are very often neither pasteurized nor filtered (at a pore size level below 1 μm), a fact that might affect the quality and safety of the produced beer since the end-product could be more subjected to microbial contamination than industrial beer (Maifreni et al., 2015; Nothaft, 2003). However, the available scientific literature does not indicate whether beers produced in microbreweries are comparable in their BA content to industrially-scale produced beers, which may have an impact on the safety of these beverages. According to Poveda et al. (2017) Spanish beers from microbreweries contained BA in an amount of about 20 mg/l. On the contrary, Choi, Lee, Sukla & Kim (2012) examined the BA content of Korean beers from microbreweries and found that most of them had a BA content below 50 mg/l, however, in more than 25% of the examined samples the total BA content was above 50 mg/l. Moreover, Pradenas et al. (2016) found higher BA content in beers from microbreweries compared to industrial-scale manufactured beers. However, a study delineating with the content of BA in beers produced in microbreweries located in the Central Europe region (a region with high incidence of microbreweries) up to now is missing.

The objective of the current work was to determine the content of BA in beers produced in microbreweries located in the Czech Republic and evaluate their safety in terms of BA occurrence.

2 Material and methods

2.1 Craft beer samples

In the period 2017 – 2018 a total number of 115 craft beer samples (packaged in glass or polyethylene terephthalate bottles) were purchased from the Central Europe marketplace (supermarket and specialized stores). Moreover, the craft beer samples were from 35 different microbreweries and the declared ethanol content of the beer samples ranged within the interval of 3.9 – 7.8 % (v/v). The examined beer samples were divided into the following 3 groups according to their extract of original wort values (**EOW**): (i) Light craft beer (LICB; EOW ≤ 10; samples 1 – 12; 12 samples in total), (ii) Lager craft beer (LACB; 11 ≤ EOW ≤ 12; samples 13 – 77; 65 samples in total), (iii) Special craft beer (SPCB; EOW ≤ 13; samples 78 – 115; 38 samples in total). In addition, 89 craft beer samples were characterized as pale (or yellow) in color, 14 samples as amber (or brown) and 12 samples as dark (or black).

For the determination of BA content from each craft beer sample 4 different batches were obtained and from each batch 4 samples were analyzed (n=16; 4 different manufacture batches × 4 samples per batch. Furthermore, 2 samples from each batch were analyzed immediately after purchase (B; at the begging of the storage period) and 2 samples from the same batch were stored at 20 ± 2 °C (in a controlled temperature chamber in the absence of sunlight and UV radiation) until the end of the best before period and subsequently were analyzed (E; at the end of the storage period). Nevertheless, the storage period of the examined craft beer samples varied and ranged from 9 to 44 days (at 20 ± 2 °C).

Prior to BA determination the pH of the craft beer samples was determined with a pH meter (Eutech Instruments, Oakton, Malaysia). Moreover, before the pH measurement the samples were shaken for 30 minutes at 20 ± 2 °C in order to remove CO_2.

2.2 Determination of biogenic amine content

Degassed (using an ultrasonic bath) craft beer samples were diluted 1:1 (v / v) with perchloric acid (c = 1.2 mol/l). The content of 8 biogenic amines (histamine - HIM, tyramine - TYM, phenylethylamine - PHE, tryptamine - TRM, putrescine - PUT, cadaverine - CAD, spermidine - SPD and spermine - SPN) was determined by liquid chromatography (LabAlliance, State College, USA; Agilent Technologies, Agilent, Paolo Alto, USA) after derivatization with dansyl-chloride. Furthermore, derivatization, chromatographic separation (column: ZORBAX Eclipse Plus C18, 50 mm × 3.0 mm, 1.8 µm, Agilent Technologies) and detection (spectrophotometrically λ = 254 nm) were performed according to Buňka et al. (2012). Each batch of craft beer was analyzed from two bottles, the samples from each bottle were derivatized three times, and each derivatized mixture was loaded onto the chromatographic column three times (n = 18). The results of the individual BA contents in the examined craft beer samples was represented by obtaining 72 (4 × 18 = 72) values in total. The limits of quantification for the individual BA were in the range 0.13 - 0.52 mg/l.

2.3 Statistical analysis

The differences between BA concentrations of the tested craft beer samples were statistically evaluated by Kruskal-Wallis and Wilcoxon tests. Kruskal-Wallis test was applied also for analysis of variance (evaluation of the effect of storage time and samples groups on

BA occurrence). Correlation analysis was also carried out using Spearman correlation coefficient. Statistical software Unistat® 5.6 (Unistat Ltd., London, UK) and the significance level of 0.05 was used for the tests.

3 Results and discussion

The results of the determination of the BA content of the tested beers samples from microbreweries of the Central Europe region are given in **Table 1**. SPD and SPN were detected in all examined beer samples, but their concentrations were below 20 mg/l at the end of the best before period. The presence of SPD and SPN can be explained by their role in nucleic acid metabolism and their presence in alcoholic beverages including beers is expected. In addition to yeast and its residues the presence of SPD and SPN can also be derived from malt (Kalač et al., 1997; 2002). Moreover, in **Table 1** is depicted that low TRM and PHE levels were also detected in 5% of the tested beer samples, however, their concentrations were <10 mg/l ($P < 0.05$). Additionally, the above-mentioned findings are in accordance to that previously reported by Almeida, Fernandes & Cunha (2012), Anli, Vural, Demiray & Mert (2006), Buňka et al. (2012), Izquierdo-Pulido et al. (1996a; 1996b), Loret et al. (2005) and Slomkowska & Ambroziak (2002),. In general, from a food safety point of view, the reported concentrations can generally be considered to be of low risk for the consumers safety.

Furthermore, in the analyzed craft beer samples immediately after purchase (B), HIM was detected (in 20% of the samples) with concentrations below 10 mg/l for 13 beer samples and within the range of 10 – 20 mg/l for 10 samples. However, at the end of the best before date, HIM was detected in 30% of the samples ($P < 0.05$), with 7 beers showing a HIM concentration of 10 – 20 mg/l and 8 samples with a HIM amount of 20 – 50 mg/l. Moreover, concentrations above 10 mg/l were detected in LACB and SPCB samples, both after purchase and at the end of the best before period. However, concentrations of HIM higher than 20 mg/l (which were detected in some LACB and SPCB samples) may present a health risk to the consumers. Moreover, a typical HIM concentration < 20 mg/l was reported in the studies of Almeida et al. (2012), Anli et al. (2006), Kalač et al. (2002), Loret et al. (2005), Pradena et al. (2016), Romero, Bagur, Sanchez-Vinas, Gásquez (2003).

Therefore, other abundant detected BA were PUT and CAD, which were detected in 90%, resp. 80% of the monitored craft beer samples. In the samples analyzed immediately after purchase, the levels of PUT and CAD were most frequently found to be <10 mg/l, or ranged within the interval of 10 – 20 mg/l (**Table 1**). Only in 4 cases (1 LACB and 3 SPCB) for CAD and in 3 cases (2 LACB and 1 SPCB) for PUT the concentration was in the range of 20 – 50 mg/l ($P < 0.05$). Moreover, with the progress of the storage period, however, the PUT and CAD content of the tested beers was significantly increased ($P < 0.05$). At the end of the best before date, 10% of the examined beer samples exceeded the PUT concentration of 20 mg/l. In particular 8 beer samples exceeded the level of 50 mg/l and one SPCB sample contained 103.1 ± 8.9 mg/l. In the case of CAD, the detected values were similar to that for PUT and at the end of the best before date the concentration in 14% of tested samples was higher than 20 mg/l. Hence, 12 beer samples reached CAD concentrations within the interval of 20 – 50 mg/l, 1 LACB and 2 SPCB 50 - 100 mg/l and in 2 SPCB the concentration of CAD was 140.5 ± 10.2 mg/l and 113.8 ± 7.6 mg/l, respectively. Generally, higher concentrations of CAD were found in LACB and SPCB samples in comparison to LICB ($P < 0.05$). The results are in agreement to those of Almeida et al. (2012), Buňka et al. (2012), Choi et al. (2012), Izquerdo-Pulido et al. (1996a; 1996b), Pradenas et al. (2016), Romero et al. (2003), Slomkowska & Ambroziak (2002),. On the other hand, in some of the above-mentioned studies, beer samples exceeding 20 mg/l or 50 mg/l of PUT or CAD content were reported, however, the proportion of such samples was around 5 – 15%, a fact that is which is in accordance to the results presented in our study. The latter amounts of PUT and CAD can be evaluated as hazardous in terms of food safety, especially since PUT and CAD could enhance the the toxic effects of other BA (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994).

Furthermore, the most frequently detected BA in all tested samples (regardless of the storage period) reporting the highest concentrations was TYM. In some samples the TYM concentrations at the beginning of the experiment were found to be <10 mg/l (for 50% of the tested samples), some samples had a concentration of 10 – 20 mg/l (for 31% of the tested samples), and some ranged in the range of 20 – 50 mg/l (19% of the tested samples). Moreover, 2 LACB and 1 SPCB samples reported concentrations above 50 mg/l immediately after purchase. However, the TYM content further increased with the prolonging of the storage time (P <0.05). Particularly, only about 25% of the tested samples showed TYM levels below 10 mg/L, 40% of the samples ranged within the interval of 10 – 20 mg/l and approximately 13% of the samples had concentration of 20 up to 50 mg/l. In addition, 20% of the tested samples had a TYM content higher than 50 mg/l (P <0.05) and concentrations above 100 mg/l (101.7 - 154.1 mg/l) were detected in 10 craft beer samples. The occurrence of TYM in beer samples was previously reported in the studies of Buňka et al. (2012), Izquerdo-Pulido (1996a; 1996b), Loret et al. (2005), Romero et al. (2003). In terms of food safety, TYM appears to be the highest health hazard among BA. The results of 8 individual BA contents in examined craft beer samples are depicted in **Table 2** (LICB samples), **Table 3** (LACB samples) and **Table 4** (SPCB samples) in relation to the progress of the storage period. From the obtained results it could be reported that the most abundant BA which was detected in LICB samples was TYR, followed by PUT. In the same token, the most frequently detected BA in LACB samples was also TYR, followed by CAD and PUT. Moreover, in the case of SPCB the most abundant BA was once again identified to be TYR followed by CAD and PUT. According to Kalač et al. (2002) TYR, HIS and CAD could be probably formed during the main fermentation process by contaminating lactic acid bacteria. Moreover, elevated levels of TYR and HIS in beer can be developed by the presence of lactic acid bacteria (mainly lactobacilli) surviving insufficient thermal treatment process. In other words, increased contents of the above-mentioned BA could signalize important deficiencies during the technological practice applied in beer manufacture (Kalač et al., 2002; Izquierdo-Pulido, Mariné-Font, & Vidal-Carou, 2000).

The results of the determination of the total content of BA (expressed as a sum of 8 BA) in the examined craft beer samples (produced in microbreweries of the Central Europe) are illustrated in **Figure 1**. At the beginning of the experiment (samples B; analyzed immediately after purchase), approximately 44% of samples reported total BA concentration higher than 20 mg/l. Furthermore, 8% of the tested samples presented a total concentration of BA above 50 mg/l. A significant increase in the BA content during storage was also identified for the total BA level ($P < 0.05$). Moreover, at the end of the best before date, about 13% of the samples had a total BA concentration between 10 to 20 mg/l and approximately 33% of the samples had a total BA amount in the range of 20 – 50 mg/l. The latter BA concentration might be considered hazardous for some consumers (for example consumers using drugs inhibiting the activity of the detoxification system) Shalaby, 1996; Silla Santos, 1996, Ten Brink, Damink, Joosten, Veld, 1990). Additionally, more than 30% of the samples (E) presented total BA concentrations in the range of 50 – 100 mg/l, resulting in a serious potential health risk for even healthy consumers in combination with alcohol. However, 18% of the craft beer had a total amount of BA higher than 100 mg/l (103.6 - 213.1 mg/l), leading to the statement that these alcoholic beverages could be characterized as hazardous for the consumers (Shalaby, 1996; Silla Santos, 1996; Ten Brink et al., 1996). When comparing the total amount of BA for industrially produced beers from the Central European countries (Czech Republic and Poland) published previously by Buňka et al. (2012) and Slomkowska & Ambroziak (2002) with our results, we could state that higher BA concentrations were found in beer samples produced in microbreweries. A response to this unfavorable fact could be stricter adherence to hygiene standards for beer production, distribution and revision of the Hazard Analysis and Critical Control Point (**HACCP**) system.

Furthermore, correlation analysis was also performed in which no significant correlation coefficients ($P \geq 0.05$) were found between the content of the 8 biogenic amines tested (or the sum of the 8 BA tested) and the ethanol content or pH value (the testes craft beer samples reported pH values in the range of 4.1 to 5.0; data not shown) of the craft beer samples. The results were in accordance to those of Buňka et al. (2012).

Conclusions

The occurrence of 8 individual BA (and their total concentration expressed as a sum) in craft beer samples (115 samples in total) produced in microbreweries of the Czech Republic was evaluated. The determination of BA content was realized in beer samples analyzed immediately after purchase and at the end of the best before date. In general, with the progress of the storage time (at 20 ± 2 °C) the concentration BA significantly increased. Moreover, concentrations of HIS above 20 mg/l were detected in LACB and SPCB samples at the end of the best before date. In addition, other abundant detected BAs were PUT and CAD. However, 8 tested craft beer samples exceeded the level of 50 mg/l of PUT. In the case of CAD, 2 samples reported values higher than 100 mg/l. Furthermore, the most frequently detected BA was TYR. Thereafter, more than 30% of the tested samples presented total BAs content in the range of 50 – 100 mg/l, which could lead to serious potential health risk for even healthy consumers. However, 18% of the craft beer samples had a total amount of BA higher than 100 mg/l, leading to the statement that these alcoholic beverages could be characterized as hazardous for the consumers. In general, a probable solution to this unfavorable fact could be stricter adherence to hygiene standards for beer production, distribution and revision of the Hazard Analysis and Critical Control Point (HACCP) system.

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Table 1. Contents of biogenic amines in the tested beer samples manufactured in Czech microbreweries (mg/l) [a]

Type of beer***	Number of samples	Time*	Contents of biogenic amines (ND/+/++/+++/++++/+++++)**							
			Histamine	Tyramine	Putrescine	Cadaverine	Tryptamine	Phenyl-ethylamine	Spermidine	Spermine
LICB	12	B	10/2/0/0/0/0	0/8/3/1/0/0	5/7/0/0/0/0	9/1/2/0/0/0	12/0/0/0/0/0	12/0/0/0/0/0	0/10/2/0/0/0	0/8/4/0/0/0
		E	8/4/0/0/0/0	0/3/4/2/2/1	3/6/1/1/0/0	6/4/1/1/0/0	12/0/0/0/0/0	10/2/0/0/0/0	0/9/3/0/0/0	0/6/6/0/0/0
LACB	65	B	57/4/4/0/0/0	0/33/17/13/2/0	11/37/15/2/0/0	17/36/11/1/0/0	64/1/0/0/0/0	64/1/0/0/0/0	0/59/6/0/0/0	0/61/4/0/0/0
		E	51/6/5/3/0/0	0/18/29/9/4/5	7/20/18/6/4/0	11/26/21/6/1/0	62/3/0/0/0/0	63/2/0/0/0/0	0/50/15/0/0/0	0/56/9/0/0/0
SPCB	38	B	25/7/6/0/0/0	0/15/17/5/1/0	5/30/1/1/0/0	9/18/8/3/0/0	38/0/0/0/0/0	38/0/0/0/0/0	0/30/8/0/0/0	0/23/15/0/0/0
		E	21/10/2/5/0/0	0/8/15/4/7/4	2/21/8/3/3/1	6/14/9/5/2/2	35/3/0/0/0/0	37/1/0/0/0/0	0/26/12/0/0/0	0/21/17/0/0/0

* Time of sampling: B – at the beginning of storage period (after purchase); E – at the end of storage period (at the end of best-before period)
** Biogenic amines contents are expressed using intervals as follows: “ND” – not detected; “+” ≤ 10 mg/l; “++” 10–20 mg/l;“+++” 20–50 mg/l; “++++” 50–100 mg/l; “+++++” ≥ 100 mg/l.
*** Light craft beer (LICB): EOW [b] ≤ 10; Lager craft beer (LACB): 11 ≤ EOW ≤ 12; Special craft beer (SPCB): EOW ≤ 13
[a] The results are expressed as number of tested samples in which the current biogenic amine was or was not detected.

[b] EOW: Extract of original wort value expressed as % w/w.

Table 2. Contents of biogenic amines (BA) in the Light craft beer (extract of original wort ≤ 10) samples manufactured in Czech microbreweries (mg/l).[a]

Sample number	TRY		PHE		PUT		CAD		HIS		TYR		SPD		SPM	
	B	E	B	E	B	E	B	E	B	E	B	E	B	E	B	E
1	ND	ND	ND	ND	ND	2.8±1.9a	ND	8.5±1.0b	ND	4.0±0.3a	2.5±0.3a	5.5±0.9c	ND	ND	8.2±0.1b	5.7±0.6c
2	ND	ND	ND	ND	ND	3.6±1.0a	1.6±0.1b	8.2±1.1c	ND	ND	1.2±0.2d	6.1±0.4d	ND	ND	1.7±0.1b	4.6±0.5a
3	ND	ND	ND	ND	ND	1.7±0.2a	1.6±0.3a	1.1±0.5a	ND	ND	2.3±0.5a	4.9±0.2b	ND	ND	2.7±0.8a	8.4±0.6c
4	ND	ND	ND	ND	1,0±0.1a	7.2±0.7b	2.1±0.6c	ND	ND	ND	3.1±0.5d	13.8±0.5e	ND	ND	5.4±0.1f	4.4±0.2g
5	ND	ND	ND	ND	1,5±0.2a	1.8±0.2a	2.1±0.6a	1.4±0.0a	ND	ND	5.1±0.7b	4.5±1.0b	ND	ND	6.8±0.5c	9.3±0.8d
6	ND	ND	ND	ND	1,5±0.1a	1.5±0.1a	2.5±0.5b	1.4±0.4a	1.1±0.2a	ND	2.6±0.1b	3.9±0.5c	ND	ND	4.8±0.2d	7.7±0.2e
7	ND	ND	ND	ND	1,2±0.1a	5.7±0.4b	ND	ND	ND	ND	1.7±0.3c	7.8±0.4d	ND	ND	5.8±0.7b	3.5±0.0e
8	ND	ND	ND	ND	1,2±0.1a	13.0±0.1b	ND	21.0±0.9c	ND	ND	3.0±0.7d	44.8±0.8e	ND	ND	9.3±1.1f	4.8±0.0g
9	ND	ND	ND	ND	1,4±0.0a	ND	2.2±0.2b	ND	ND	ND	3.1±0.7c	2.3±0.3b	ND	ND	8.3±0.5d	5.6±0.1e
10	ND	ND	ND	4.6±0.1a	ND	70.2±2.4b	10.3±0.1c	ND	ND	ND	28.9±0.6d	37.0±1.5e	ND	ND	3.8±0.1f	ND
11	ND	ND	ND	ND	9,9±0.5a	5.3±0.4b	ND	ND	ND	ND	2.6±0.2c	23.6±1.8d	ND	ND	ND	8.6±0.2e
12	ND	ND	ND	ND	6,9±0.1a	11.6±0.7b	27.5±0.5c	ND	ND	ND	45.1±1.5d	51.5±3.6e	ND	ND	2.8±0.0f	3.6±0.1g
Total BA	ND	ND	ND	4.6±0.1	24,6±1.2	124.4±8.1	49.9±2.9	41.6±3.9	1.1±0.2	4.0±0.3	101.2±6.3	205.7±11.9	ND	ND	59.6±4.2	66.2±3.3

[a] The values are expressed as means ± standard deviation (n=18; each batch of craft beer was analyzed from two bottles, the samples from each bottle were derivatized three times, and each derivatized mixture was loaded onto the chromatographic column three times). The means within a line (the difference between individual BA) followed by different letters differ (P < 0.05).

[*] TRY: tryptamine; PHE: phenylethylamine; PUT: putrescine; CAD: cadaverine; HIS: histamine; TYR: tyramine; SPD: spermidine; SPM: spermine.

[**] Time of sampling: B – at the beginning of storage period (after purchase); E – at the end of storage period (at the end of best-before period).

[***] ND – not detected.

Table 3. Contents of biogenic amines (BA) in the Lager craft beer (11 ≤ extract of original wort ≤ 12) samples manufactured in Czech microbreweries (mg/l).[a]

Sample number	TRY*		PHE		PUT		CAD		HIS		TYR		SPD		SPM	
	B**	E**	B	E	B	E	B	E	B	E	B	E	B	E	B	E
13	ND***	ND	ND	ND	ND	1.2±0.0a	ND	1.2±0.5a	ND	17,5±3.1b	ND	20.4±2.0b	ND	ND	ND	4.3±0.3c
14	ND	ND	ND	2.3±0.3a	2.8±0.3a	1.9±0.1b	61.1±5.0c	57.6±3.8d	27.8±2.1e	37,1±2.4f	53,3±3.8d	59.8±3.0d	ND	ND	1.1±0.4g	ND
15	2.3±0.3a	2.9±0.0b	ND	ND	5.1±0.5c	3.0±0.1b	65.4±4.2d	53.7±4.0e	36.6±3.0f	44.6±6.1g	58.4±0.8e	71.3±7.3h	ND	ND	ND	1.0±0.2i
16	ND	ND	ND	ND	3.5±0.1a	53.8±2.8b	37.3±1.2c	27.7±2.2d	22.8±2.0e	ND	36.3±3.5c	69.0±5.6f	ND	ND	3.4±0.6a	3.3±0.1a
17	ND	ND	ND	ND	ND	17.1±0.3a	1.1±0.2b	4.6±0.1c	ND	3.9±0.4d	1.6±0.2e	1.0±0.1b	ND	ND	7.6±1.0f	4.4±0.1d
18	ND	ND	ND	ND	ND	40.6±2.1a	10.4±1.0b	13.9±0.9c	ND	ND	2.3±1.0d	6.1±0.1e	ND	ND	9.8±0.8f	4.2±0.1g
19	ND	ND	ND	ND	ND	ND	1.7±0.1a	ND	ND	ND	5.6±2.7b	ND	ND	ND	11.8±1.7c	ND
20	ND	ND	ND	ND	ND	ND	40.6±4.7a	ND	ND	ND	1.8±0.0b	ND	ND	ND	7.7±0.6c	ND
21	ND	ND	ND	ND	ND	ND	37.3±0.6a	25.7±0.2b	ND	ND	2.4±0.2c	4.8±0.8d	ND	ND	8.7±1.0e	12.8±0.6f
22	ND	ND	ND	ND	ND	1.7±0.1a	5.5±0.7b	4.8±0.6b	ND	ND	4.9±3.6b	60.4±0.7c	ND	ND	7.3±4.4b	ND
23	ND	ND	ND	ND	ND	1.4±0.1a	ND	1.8±0.9a	ND	ND	2.5±0.7a	4.4±0.3b	ND	ND	8.9±1.1c	6.7±2.4c
24	ND	ND	ND	ND	ND	1.7±0.2a	ND	2.9±1.9a	ND	ND	5.7±2.8a	4.3±1.8a	ND	ND	11.3±0.4b	7.4±0.7a
25	ND	ND	ND	ND	1.0±0.2a	1.5±0.1b	1.2±0.1a	1.7±0.3b	ND	ND	4.8±1.2c	4.9±0.6c	ND	ND	12.0±1.8d	8.3±0.3e
26	ND	ND	ND	ND	1.0±0.1a	ND	2.8±0.8b	3.1±0.1c	ND	ND	1.3±0.3d	2.2±0.3b	ND	ND	6.4±0.5e	7.4±0.5e
27	ND	ND	ND	ND	ND	7.4±0.7a	15.4±0.2b	10.0±0.8c	ND	ND	8.4±0.5a	57.5±4.7d	ND	ND	10.7±1.5c	8.8±0.2a
28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.3±0.3a	ND	ND	ND	1.8±0.0b	ND
29	ND	ND	ND	ND	ND	ND	10.6±1.3a	10.2±1.3a	ND	ND	8.7±0.8a	12.0±2.0a	ND	9.3±0.1a	3.7±0.6b	ND
30	ND	ND	ND	ND	1.2±0.1a	ND	1.0±0.3a	1.5±0.6a	ND	1.4±0.1a	2.9±0.5b	1.7±0.0a	ND	ND	9.3±0.6c	ND
31	ND	ND	ND	ND	1.0±0.1a	ND	1.1±0.1a	ND	ND	1.0±0.2a	3.5±0.6b	ND	ND	ND	12.7±1.7c	ND
32	ND	ND	ND	ND	1.1±0.1a	1.1±0.1a	31.8±1.6b	39.3±4.1c	ND	ND	12.3±1.2d	13.8±1.4d	ND	ND	4.9±0.3e	6.8±1.1f
33	ND	ND	ND	ND	ND	1.1±0.1a	23.0±1.4b	50.3±3.2c	ND	2.5±0.1d	6.6±1.3e	18.5±1.6f	ND	ND	ND	5.9±0.8e
34	ND	ND	ND	ND	ND	1.8±0.5a	ND	5.1±3.9a	ND	1.5±1.6a	0.8±0.1a	5.3±2.7a	ND	ND	1.7±0.2a	6.4±0.5a

35	ND	ND	1.7±0.4a	1.0±0.2b	ND	1.7±0.5b	22.9±1.2c	32.2±3.1d	ND	8.1±0.8e	50.8±2.4f	53.6±4.2f	ND	ND	ND	ND
36	ND	ND	ND	ND	ND	48.3±2.3a	37.1±3.4b	17.6±0.2c	ND	ND	10.9±0.4d	34.9±3.3b	ND	ND	ND	4.4±0.2e
37	ND	ND	ND	ND	ND	4.5±0.1a	30.9±1.9b	16.2±0.2c	3.8±0.8d	ND	36.2±1.2e	58.4±1.2f	ND	ND	ND	ND
38	ND	ND	ND	ND	ND	1.5±0.1a	1.9±0.2b	ND	ND	ND	2.7±0.9b	5.4±0.8c	ND	ND	3.8±0.2c	10.4±1.4d
39	ND	ND	ND	ND	ND	1.5±0.0a	29.7±1.1b	43.8±0.8c	ND	ND	3.3±1.1d	3.8±0.1d	ND	ND	2.8±0.1d	7.4±1.5e
40	ND	ND	ND	ND	ND	1.6±0.3a	30.3±1.8b	44.2±0.7c	ND	ND	2.0±0.5a	6.1±1.5d	ND	ND	2.6±0.8a	8.7±0.4e
41	ND	ND	ND	ND	1.1±0.0a	11.8±0.4b	38.9±0.0c	21.1±1.4d	6.1±1.2e	11.1±1.2b	23.0±2.8d	23.5±1.8d	ND	ND	1.3±0.5a	2.7±0.1e
42	ND	ND	ND	ND	ND	1.1±0.1a	ND	ND	ND	ND	1.5±0.2b	4.1±2.3c	ND	ND	2.0±0.6c	7.2±0.7d
43	ND	ND	ND	ND	ND	1.0±0.1a	ND	ND	ND	ND	1.9±0.5b	3.8±2.3b	ND	ND	2.0±0.7b	7.3±0.4c
44	ND	ND	ND	ND	1.2±0.0a	16.3±0.1b	6.2±0.7c	43.8±1.0d	ND	ND	10.4±0.7e	12.1±0.2f	ND	ND	1.2±0.2a	4.3±0.2g
45	ND	ND	ND	ND	ND	1.4±0.3a	2.5±0.5b	16.2±1.5c	ND	5.7±0.6d	9.0±0.2e	11.2±1.3f	ND	1.0±0.1a	1.6±0.5a	9.0±0.4e
46	ND	ND	ND	3.8±1.1a	ND	1.1±0.1b	2.1±0.2b	1.6±0.6b	ND	ND	2.9±1.4b	3.8±0.9b	ND	ND	3.1±0.7b	9.7±0.8c
47	ND	ND	ND	1.4±0.3a	ND	1.4±0.1a	1.6±0.2a	1.6±0.6a	ND	1.2±0.1a	2.6±0.7a	6.4±3.1b	ND	ND	4.9±1.2b	9.0±0.3b
48	ND	ND	ND	ND	ND	ND	38.1±2.0a	44.8±0.8b	ND	ND	36.3±2.4a	28.8±0.6c	ND	ND	ND	1.3±0.1d
49	ND	ND	ND	ND	ND	ND	36.1±2.6a	38.8±4.0a	ND	ND	35.7±0.3a	24.4±1.1b	ND	ND	1.5±0.7c	1.4±0.6c
50	ND	1.8±1.3a	2.5±0.4a	2.0±0.5a	2.3±0.2a	2.0±0.1a	75.5±4.3b	61.7±3.8c	ND	36.2±5.5d	44.4±4.2e	50.3±6.5e	ND	ND	ND	ND
51	ND	ND	2.6±0.5a	1.0±0.0b	2.4±0.4a	2.0±0.2a	71.5±1.8c	60.0±1.5d	ND	29.3±1.1e	43.2±2.2f	ND	ND	ND	ND	ND
52	ND	ND	1.1±0.3a	1.0±0.4a	2.0±0.7a	1.7±0.1a	62.5±5.5b	61.1±1.4b	ND	ND	30.7±5.7c	ND	ND	ND	ND	ND
53	ND	ND	2.0±1.2a	ND	2.2±0.5a	11.7±1.0b	68.5±5.2c	31.2±2.2d	ND	ND	39.0±5.2d	55.7±4.1e	ND	ND	ND	ND
54	ND	ND	ND	ND	1.6±0.1a	1.5±0.3a	59.8±1.9b	56.7±9.5b	ND	ND	42.9±3.0c	ND	ND	ND	ND	ND
55	ND	ND	ND	ND	2.5±0.7a	ND	6.5±4.7a	ND	ND	ND	4.5±2.7a	ND	ND	ND	1.8±0.8a	2.6±0.7a
56	ND	ND	ND	ND	1.3±0.2a	ND	2.4±0.4b	ND	ND	ND	5.4±0.0c	1.3±0.1a	ND	ND	11.1±0.9d	3.7±0.2e
57	ND	ND	ND	ND	1.2±0.4a	1.6±0.1a	1.6±0.4a	1.2±0.1a	ND	ND	6.2±3.2b	2.7±0.2c	ND	ND	5.9±0.8b	6.2±0.5b
58	ND	ND	ND	ND	1.1±0.0a	17.2±1.0b	1.6±0.1c	42.0±4.8d	ND	23.9±4.5e	6.7±0.1f	113.6±10.4h	ND	ND	5.1±0.4i	62.9±7.1j
59	ND	ND	ND	ND	1.4±0.0a	11.8±1.0b	1.8±0.0c	3.4±0.0d	ND	ND	6.2±1.5e	3.4±0.3d	ND	ND	9.0±0.9f	9.2±0.4f
60	ND	ND	ND	ND	1.2±0.3a	1.4±0.1a	1.9±0.8a	1.0±0.4a	ND	ND	41.9±1.7b	42.8±1.6b	ND	ND	2.3±0.6a	3.5±0.3c
61	ND	ND	ND	ND	1.5±0.1a	1.6±0.1a	2.8±0.4b	1.2±0.2a	ND	ND	4.1±1.0c	15.4±2.1d	1.1±0.0a	ND	6.0±1.1e	10.0±0.4f
62	ND	ND	ND	ND	ND	5.3±0.3a	ND	ND	ND	ND	5.0±0.8a	12.0±0.5b	ND	ND	4.7±1.1a	4.0±0.1a
63	ND	ND	ND	ND	1.3±0.0a	6.0±0.4b	1.7±1.2a	ND	ND	ND	2.5±0.8a	7.8±2.4b	ND	ND	4.9±0.4b	3.2±0.2c

64	ND	ND	ND	ND	1.2±0.1a	5.6±0.5b	ND	2.2±0.1c	ND	ND	1.7±0.3d	11.6±1.0e	ND	ND	5.5±0.7b	3.4±0.1f
65	ND	ND	6.3±4.8a	ND	6.6±1.2a	1.9±0.5b	25.7±3.5c	ND	15.3±1.5d	ND	16.6±1.3d	5.0±1.6a	ND	ND	8.3±3.2a	8.7±0.9a
66	ND	ND	ND	ND	5.6±0.3a	33.0±0.3b	48.9±2.3c	19.9±0.3d	27.4±1.1e	ND	17.4±1.0f	13.9±0.4g	ND	ND	6.2±0.5a	5.5±0.2a
67	ND	ND	ND	ND	1.8±0.2a	36.1±3.8b	43.4±0.9c	19.2±1.7d	ND	ND	7.3±1.2e	69.9±4.1f	ND	ND	6.1±0.7e	3.5±0.1g
68	ND	ND	3.3±0.2a	ND	2.7±2.2a	1.4±0.0a	12.4±1.5b	1.6±0.2a	10.8±0.5b	ND	8.4±1.7b	7.4±2.9b	ND	ND	8.1±0.7b	9.4±0.8b
69	ND	ND	2.5±1.3a	ND	3.1±0.2a	1.7±0.1a	91.5±7.0b	2.7±0.2a	ND	1.5±0.0a	59.0±0.7c	7.1±1.9d	ND	ND	ND	8.4±0.7d
70	ND	ND	ND	ND	ND	16.2±1.9a	20.5±0.9b	21.6±1.5b	12.4±0.2c	ND	28.5±1.5d	45.5±3.6e	ND	ND	ND	ND
71	ND	ND	ND	ND	18.2±0.6a	10.6±0.6b	13.1±1.1c	ND	ND	ND	3.9±1.1d	14.9±1.3c	ND	ND	6.7±0.0e	7.6±0.5f
72	ND	ND	ND	ND	15.7±0.9a	22.5±0.3b	ND	ND	ND	ND	5.8±0.4c	17.2±1.2d	1.7±0.0e	ND	ND	7.3±0.3f
73	ND	ND	ND	ND	15.0±1.5a	16.4±0.3a	3.3±0.1b	ND	ND	ND	ND	3.3±0.5b	6.9±0.2c	ND	7.4±0.3c	ND
74	ND	ND	ND	ND	9.9±0.4a	15.0±1.5b	20.7±0.5c	ND	ND	ND	72.5±2.2d	84.1±2.1e	ND	ND	ND	ND
75	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	24.2±3.6a	46.9±3.4b	ND	ND	4.9±0.2c	3.7±0.1d
76	ND	ND	ND	ND	11.5±0.7a	5.2±0.7b	ND	ND	ND	ND	ND	47.8±0.9c	ND	ND	8.9±0.1d	4.7±0.1b
77	ND	ND	ND	ND	15.4±1.7a	25.7±0.5b	ND	ND	ND	ND	ND	9.9±1.2c	ND	ND	12.6±1.0d	11.3±0.0e
Total BA	2.3±0.3	4.7±1.3	22.0±9.1	12.5±2.8	147.7±15.0	483.6±25.7	1263.2±87.8	1023.7±71.0	163.0±12.4	226.5±27.8	985.6±87.2	1387.2±110.5	9.7±0.2	10.3±0.2	293.1±30.4	349.3±25.8

[a] The values are expressed as means ± standard deviation (n=18; each batch of craft beer was analyzed from two bottles, the samples from each bottle were derivatized three times, and each derivatized mixture was loaded onto the chromatographic column three times). The means within a line (the difference between individual BA) followed by different letters differ (P < 0.05).

[*] TRY: tryptamine; PHE: phenylethylamine; PUT: putrescine; CAD: cadaverine; HIS: histamine; TYR: tyramine; SPD: spermidine; SPM: spermine.

[**] Time of sampling: B – at the beginning of storage period (after purchase); E – at the end of storage period (at the end of best-before period).

[***] ND – not detected.

Table 4. Contents of biogenic amines (BA) in the Special craft beer (extract of original wort ≤ 13) samples manufactured in Czech microbreweries (mg/l).[a]

Sample number	TRY		PHE		PUT		CAD		HIS		TYR		SPD		SPM	
	B	E	B	E	B	E	B	E	B	E	B	E	B	E	B	E
78	1.1±0.0a	ND	5.2±0.4b	1.4±0.7a	ND	ND	39.0±3.2c	33.0±1.8c	7.6±0.6d	15.3±0.7e	49.3±4.2f	89.3±2.8g	ND	ND	ND	1.1±0.8a
79	ND	ND	ND	ND	ND	ND	1.7±0.5a	ND	ND	49.2±2.4b	3.7±0.6c	12.2±0.9d	ND	1.7±0.0a	12.5±3.0d	ND
80	ND	ND	ND	2.2±0.6a	ND	1.9±0.4a	1.0±0.2b	1.8±0.1a	ND	8.2±0.9c	3.4±0.4d	11.7±1.3e	ND	ND	11.3±1.2e	8.0±0.5c
81	ND	ND	ND	ND	1.0±0.2a	1.6±0.3a	10.9±1.6b	17.0±2.2c	1.4±0.2a	3.6±0.4d	15.3±2.7c	19.4±3.4c	ND	ND	10.8±0.2b	9.2±1.0b
82	ND	4.3±0.4a	ND	2.1±0.7b	1.1±0.1c	1.2±0.3c	1.1±0.2c	2.7±0.3b	0.1±0.0d	ND	5.0±0.8a	63.8±6.3e	ND	ND	10.2±0.0f	ND
83	ND	ND	ND	ND	1.0±0.1a	1.2±0.0a	1.6±0.3a	ND	ND	ND	ND	7.4±1.0b	ND	ND	11.8±1.2c	16.0±2.0d
84	ND	ND	ND	ND	ND	1.1±0.1a	21.5±0.9b	28.6±1.3c	ND	ND	24.5±0.7d	26.8±1.6d	ND	3.9±0.0e	2.1±0.1f	ND
85	ND	ND	ND	ND	ND	1.0±0.1a	25.8±0.0b	38.3±0.5c	ND	ND	25.1±2.7b	27.7±3.7b	ND	4.5±0.3d	1.8±0.4e	ND
86	ND	ND	ND	ND	1.0±0.4a	ND	ND	ND	ND	ND	7.3±0.3b	14.1±1.4c	ND	38.7±1.0d	12.8±0.9c	ND
87	ND	ND	ND	ND	1.4±0.2a	2.0±0.2b	17.7±2.0c	27.7±0.8d	ND	ND	40.7±8.1e	48.3±0.4e	ND	ND	2.7±0.6b	2.0±0.0b
88	ND	ND	ND	ND	1.0±0.4a	ND	1.7±0.8a	1.0±0.1a	ND	ND	3.8±1.2b	7.1±0.2c	ND	ND	11.9±1.0d	16.0±1.6e
89	ND	ND	ND	ND	ND	1.0±0.0a	ND	1.2±0.5a	ND	10.2±0.3b	1.4±0.1a	5.5±0.7c	ND	ND	ND	9.4±0.3d
90	ND	ND	ND	ND	ND	2.1±0.2a	2.8±0.4a	2.0±0.5a	ND	ND	ND	4.5±0.2b	ND	ND	ND	7.8±1.2c
91	ND	ND	ND	ND	ND	1.8±0.1a	45.1±5.0b	64.3±7.3c	7.4±2.1d	ND	6.9±0.5d	11.4±2.2e	ND	1.0±0.1f	4.4±0.9g	10.6±1.1e
92	ND	ND	1.2±0.4a	ND	ND	1.3±0.1a	43.7±2.3b	43.0±1.1b	ND	ND	50.1±4.8b	53.2±4.8b	ND	ND	ND	ND
93	ND	ND	1.6±0.5a	1.7±0.4a	ND	2.9±0.4b	7.7±0.4c	8.7±0.0d	ND	ND	54.0±1.7e	60.9±6.9e	ND	ND	ND	ND
94	ND	ND	1.8±0.4a	ND	ND	6.6±0.3b	28.3±1.0c	36.1±1.9d	ND	ND	44.9±3.5e	47.3±3.1e	ND	ND	ND	ND
95	ND	ND	ND	ND	ND	14.7±1.8a	34.9±4.7b	15.7±1.1a	ND	ND	ND	21.7±1.6d	ND	ND	ND	4.3±0.1e
96	ND	ND	2.9±0.5a	3.1±0.3a	1.6±0.3b	4.9±0.3c	62.6±1.8d	21.3±4.6e	ND	ND	74.6±6.4	4.2±0.3c	ND	ND	1.1±0.0b	2.2±0.8a
97	ND	ND	ND	ND	1.2±0.2a	2.1±0.1b	44.9±4.9c	70.3±3.3d	ND	ND	45.4±1.6c	47.8±1.3c	ND	ND	1.3±0.3a	1.6±0.0a
98	ND	ND	ND	ND	1.0±0.1a	8.1±0.3b	2.9±0.4c	11.3±0.1d	ND	ND	73.7±0.8e	80.0±6.7e	ND	ND	3.4±0.4c	4.3±0.1c
99	ND	ND	2.3±0.5a	1.6±0.5a	1.6±0.4a	ND	16.5±1.7b	8.5±0.7c	ND	ND	44.1±4.9d	38.9±5.9d	ND	ND	ND	ND
100	ND	ND	ND	ND	2.1±0.0a	1.7±0.3a	1.4±0.2a	2.5±0.4a	ND	30.2±0.8b	2.0±0.1a	ND	ND	ND	1.0±0.3a	2.6±0.8a
101	ND	ND	1.4±0.6a	ND	2.7±0.1b	2.3±0.3b	ND	3.5±0.6c	30.2±1.1d	29.7±0.4d	38.2±4.5e	40.9±2.2e	ND	ND	ND	1.3±0.1a
102	ND	ND	ND	ND	1.6±0.1a	1.8±0.0a	1.5±0.4a	1.6±0.4a	25.7±0.7b	30.1±0.4c	9.4±0.3d	13.0±0.3e	ND	ND	2.3±0.9f	5.1±0.2g

103	ND	ND	2.5±2.2a	ND	1.3±0.2b	1.2±0.1b	4.5±0.0c	3.9±0.5c	3.1±0.3c	5.8±0.1d	3.8±0.0c	29.7±0.6e	ND	ND	8.6±1.7f	1.2±0.1b
104	ND	ND	ND	ND	1.9±0.1a	1.6±0.2a	58.0±3.0b	47.4±1.9c	2.8±0.4d	ND	6.8±0.4e	7.5±0.7e	ND	ND	6.2±0.5e	2.3±0.3f
105	ND	ND	ND	ND	1.3±0.1a	24.6±0.7b	ND	ND	ND	ND	2.8±0.2c	21.7±1.1d	ND	ND	8.3±0.7e	7.7±0.3e
106	ND	ND	ND	ND	1.1±0.1a	1.2±0.3a	35.2±0.6b	41.9±6.1b	ND	ND	22.9±2.5c	33.3±6.6d	ND	3.2±0.5e	2.5±0.4f	ND
107	ND	ND	ND	ND	1.9±0.2a	10.8±1.0b	1.8±0.4a	ND	ND	ND	5.1±0.6c	46.3±1.9d	ND	ND	12.9±2.1e	11.4±0.1e
108	ND	ND	ND	ND	1.8±0.1a	1.7±0.4a	23.2±2.9b	17.4±0.7c	ND	ND	12.5±1.8d	12.0±5.3d	32.7±0.2e	ND	ND	14.7±1.1d
109	ND	ND	ND	ND	2.6±0.5a	11.0±0.9b	5.5±0.1c	4.4±0.2d	12.7±0.2b	ND	11.8±1.6b	7.1±0.3e	ND	ND	4.3±0.8d	5.4±0.2d
110	ND	ND	ND	ND	2.0±0.1a	8.6±0.1b	3.7±1.1c	2.3±0.1a	1.5±0.1d	ND	6.1±0.5e	44.3±0.7f	ND	ND	9.6±1.5f	9.7±0.5f
111	ND	ND	ND	ND	1.6±0.1a	1.6±0.0a	1.1±0.1b	1.4±0.1a	ND	ND	7.0±1.8c	5.5±0.0d	ND	ND	10.4±2.1e	8.1±0.1f
112	ND	ND	ND	ND	1.5±0.2a	89.8±4.5b	33.0±5.3c	14.5±1.9d	ND	ND	6.9±0.5e	10.6±0.9d	ND	ND	14.3±1.6d	11.0±0.4d
113	ND	ND	ND	ND	ND	15.8±2.4a	ND±	ND	ND	ND	2.1±0.2b	ND	ND	ND	14.4±1.0a	15.2±1.4a
114	ND	ND	ND	ND	11.3±0.2a	15.0±1.0b	ND±	ND	ND	ND	1.7±0.1c	30.9±2.6d	ND	ND	ND	ND
115	ND	ND	ND	ND	7.3±0.5a	119.1±3.0b	3.2±0.1c	ND	ND	ND	8.2±1.2d	17.5±2.0e	ND	ND	4.4±0.4f	4.8±0.2f
Total BA	1.1±0.0	4.3±0.4	23.2±5.5	16.7±3.2	53.9±5.0	363.3±20.0	583.5±46.7	573.3±41.1	92.5±5.7	182.3±6.4	720.5±62.3	1023.5±81.9	32.7±0.2	53.0±1.9	197.3±46.7	192.9±15.3

[a] The values are expressed as means ± standard deviation (n=18; each batch of craft beer was analyzed from two bottles, the samples from each bottle were derivatized three times, and each derivatized mixture was loaded onto the chromatographic column three times). The means within a line (the difference between individual BA) followed by different letters differ ($P < 0.05$).

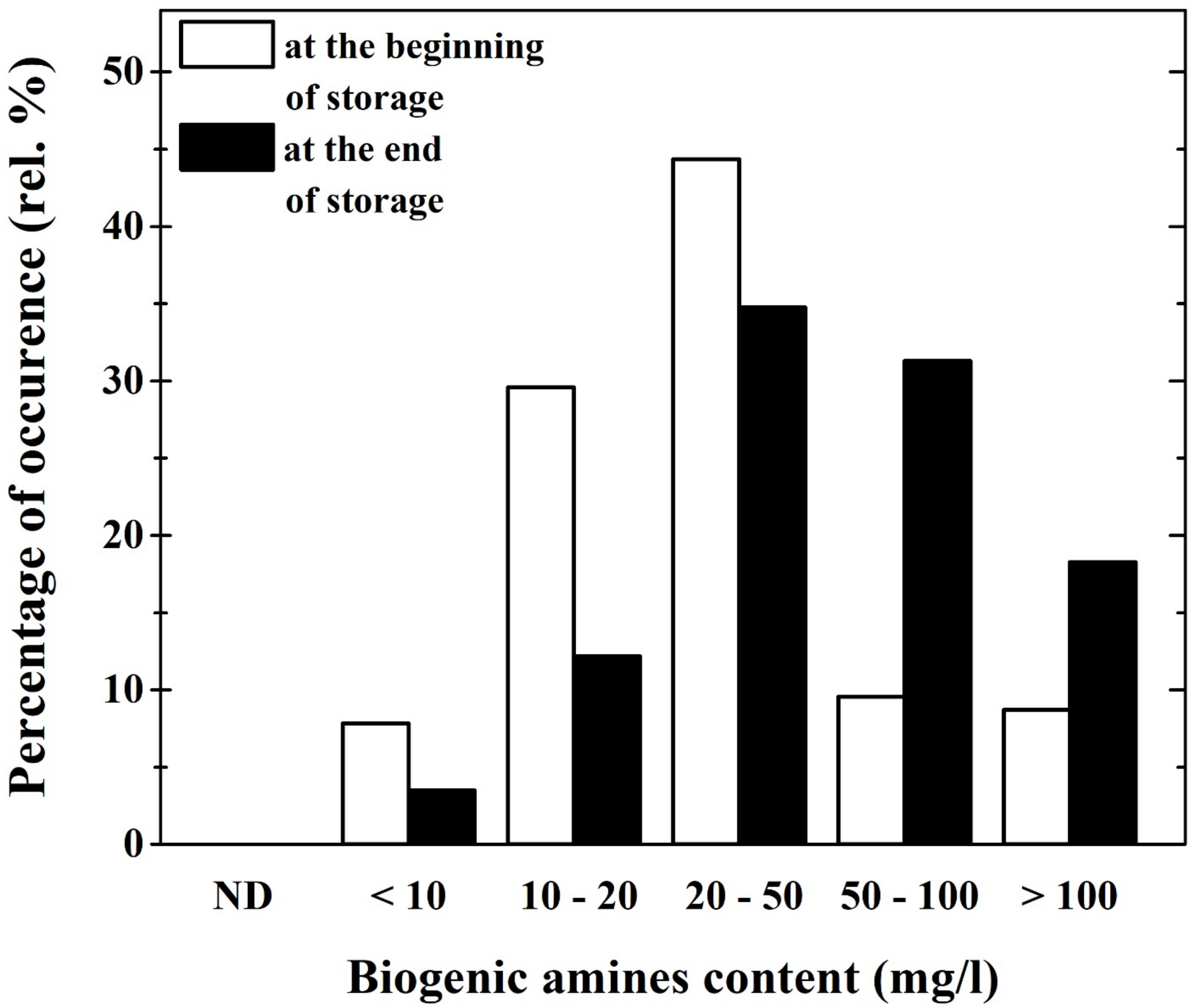
[*] TRY: tryptamine; PHE: phenylethylamine; PUT: putrescine; CAD: cadaverine; HIS: histamine; TYR: tyramine; SPD: spermidine; SPM: spermine.

[**] Time of sampling: B – at the beginning of storage period (after purchase); E – at the end of storage period (at the end of best-before period).

[***] ND – not detected.

Figure 1

The occurrence of the total amount of biogenic amines (mg/l) in beers produced in Czech microbreweries at the beginning of storage (white columns) and at the end of storage (black columns). The results are expressed as percentage of total amount of beers tested (115 samples); ND = not detected.



Highlights:

- Biogenic amine occurrence in Czech craft beers was monitored.
- With the progress of the storage the biogenic amine content increased.
- The most frequently detected biogenic amine was tyramine.
- 18% of the beers presented biogenic amine values higher than 100 mg/l.

Conflict of interest Form

Dear Editors,

We would like to submit the enclosed manuscript entitled "*Biogenic amines occurrence in beers produced in Czech microbreweries*", which we wish to be considered for publication in "Food Control". Moreover, no conflict of interest exits in the submission of this manuscript, and the manuscript is approved by all authors for publication. I would like to declare on behalf of my coauthors that the work described was original research that has not been published previously, and not being under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

Thank you and best regards.

Yours sincerely,

Richardos Nikolaos Salek