



Assessment of biogenic amines profile in ciders from the Central Europe region as affected by storage time

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ABSTRACT

The aim of the research was the assessment of 8 biogenic amines occurrence (BA) in ciders. Forty samples with ethanol content $\leq 4.5\%$ v/v (LC; low alcohol content) and 34 samples with ethanol content $> 4.5\%$ v/v (HC; high alcohol content) manufactured in the Central Europe region were tested. The cider samples were immediately analyzed after purchase and at the end of the best before date. The most abundant BA across all ciders was tyramine, followed by putrescine and cadaverine ($p < 0.05$). Samples reported low levels of tryptamine, spermidine, spermine, histamine and phenylethylamine. No cider at the end of the best before date had a sum of BA below $< 5 \text{ mg l}^{-1}$. Moreover, 67% of the samples at the beginning of the storage period and 47% of the ciders after the best before date presented a total BA content in the range of $5\text{--}20 \text{ mg l}^{-1}$. A total number of 14% of the samples immediately after purchase and 31% of samples at the end of the best before date showed a BA concentration in the range of $20\text{--}50 \text{ mg l}^{-1}$. The BA content of 16 samples was $> 50 \text{ mg l}^{-1}$ at the end of the best before date. However, one LC cider and two HC products displayed a BA sum of nearly 120 mg l^{-1} . In general, higher concentrations of tyramine, cadaverine and putrescine were detected in LC samples. All in all, with the prolonging of storage the BA concentration increased.

1. Introduction

Biogenic amines (BA) are low molecular-weight nitrogenous bases that can cause human health problems (e.g. headaches, vomiting, cardiac palpitations, hypotension or hypertension, flushing and respiratory problems) when taken in large amounts or are restrained by the detoxification mechanisms of the human body (primarily through monoamine oxidases, diamino-oxidases, and histidine methyltransferases). In general, BA are found in plethora of food and beverages, commonly associated with products whose elaboration involves ripening or a fermentation process. The most important BA present in foods are histamine, tyramine, putrescine, cadaverine, tryptamine, 2-phenylethylamine, spermine, spermidine, and agmatine (Burgut et al., 2020; del Rio et al., 2019, 2017; Garai et al., 2006). However, in case of excessive BA intake, the detoxification mechanism may be insufficient and the health of the consumer may be endangered and, in extreme cases, this may even lead to death. Generally, the upper limit threshold

of total BA intake is 1000 mg kg^{-1} . However, the latter limit is consumer dependent. Concentrations of individual BA up to 100 mg kg^{-1} and/or 100 mg l^{-1} , respectively, could be considered as safe. However, various compounds such as ethanol and various medicaments can significantly reduce the effectiveness of the detoxification mechanism. Therefore, the recommended limits for alcoholic beverages are much lower and in the case of some BA it can be units of milligrams per liter (BIOHAZ, 2011; Coton et al., 2010; Jia et al., 2020; Pradenas et al., 2016; Tofalo et al., 2016, p. 424).

BA are usually formed by decarboxylation of free amino acids. Decarboxylases are naturally present in plant and animal cells, and microorganisms can produce BA for their metabolic processes (e.g. many strains of lactic acid bacteria, Enterobacteriaceae, *Pseudomonas* spp. etc.). Excessive concentrations of BA in food can lead to food poisoning. According to their chemical structure, BA can be divided into the following three main groups: (i) aliphatic – putrescine (PUT), cadaverine (CAD), spermine (SPN), spermidine (SPD) and agmatine (AGM); (ii)

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aromatic – tyramine (TYM) and phenylalanine (PHE); and (iii) heterocyclic – histamine (HIM) and tryptamine (TRM). BA occur both in (i) fermented food (usually produced by starter cultures); as well as in (ii) unfermented food (mainly formed by the action of contaminating microorganisms). Although BA production by contaminating bacteria found in starter preparations used in winemaking has been reported, starter cultures must be selected taking into account the absence of genetic determinants for BA production. Moreover, in the case of fermented foodstuff, contaminating microflora may also be present and as a consequence the formation of BA might be realized (Barbieri et al., 2019; Costantini et al., 2009; Halász et al., 1999; Houicher et al., 2021; Russo et al., 2017; Spano et al., 2010; Tofalo et al., 2016, p. 424).

Cider is a slightly alcoholic beverage (ethanol content in the range of 1.2–8.0%, v/v) produced via apple (*Malus domestica*) must or reconstituted apple juice fermentation process (partial or complete). Nowadays, selected strains of *Saccharomyces cerevisiae* or *Saccharomyces bayanus* are applied to accomplish the appropriate alcoholic fermentation in order to minimize the formation of substances which could lead to organoleptic deficiencies of the final product. However, some traditionally manufactured ciders are purposely produced through apple juice spontaneous fermentation carried out by indigenous microflora (mainly yeasts). Furthermore, malolactic fermentation (MLF) is a secondary biological fermentation (realized after alcoholic fermentation) attended by lactic acid bacteria (LAB). During MLF L-malic acid is transformed (via malolactic enzyme) into L-lactic acid and CO₂, affecting the sensory attributes of cider. However, cider is a product, in which LAB are very often responsible for the production of BA. The main BA-producing LAB include strains of the genera *Oenococcus*, *Lactobacillus*, *Pediococcus*, *Enterococcus* (Buňková et al., 2009; Costantini et al., 2013; Li et al., 2020; Lorencová et al., 2012; Lorenzini et al., 2019; Perpetuini et al., 2020; Wei et al., 2020).

The study of Ladero et al. (2011) provided information about the occurrence of BA in commercially available ciders from Spain and France. TRM, PHE, SPD and SPN were represented in minority. On the other hand, in the above-mentioned study the occurrence of HIM, TYM, PUT and CAD was reported in concentrations > 20 mg/l, which in connection with ethanol intake can cause health problems even in healthy individuals. Furthermore, slightly lower concentrations of BA in Spanish ciders were reported by Garai-Ibabe et al. (2013). However, in alcoholic beverages (including beer, wine and cider) the toxicity of BA is enhanced by the presence of ethanol, being an inhibitor of mono amino oxidases (Gardini et al., 2016; Perpetuini et al., 2020).

The production of cider is growing throughout Europe and gradually is spreading all over the world (Rosend et al., 2019). However, the occurrence of BA in ciders produced and sold in Central Europe has not been up to now monitored in the available scientific literature. The objective of the current study was to determine the content of BA in ciders manufactured in the Central Europe region and to evaluate final product safety in terms of BA occurrence.

2. Material and methods

2.1. Chemicals and reagents

Biogenic amine standards [histamine dihydrochloride 99% (purity), tyramine hydrochloride 97%, phenethylamine hydrochloride 99%, tryptamine hydrochloride 98%, putrescine dihydrochloride 99%, cadaverine dihydrochloride 99%, spermidine trihydrochloride 99.5%, spermine tetrahydrochloride 99.5%], benzoyl chloride, proline, heptane, acetonitrile, 1,7-diaminoheptane, Na₂CO₃, K₂CO₃, acetone NaHCO₃ and perchloric acid were purchased from SigmaAldrich Inc. (St. Louis, MO). All chemicals used were of analytical reagent grade or higher.

2.2. Cider samples

During 2018 and 2019, a total amount of 74 cider samples (32 different producers) were obtained from retail stores located in the Central Europe region (including Austria, Czech Republic, Germany, Slovakia and Poland). All cider samples were pasteurized (information from the vignettes/package provided by the manufacturer). The purchased cider samples were categorized into two main groups according to their ethanol content: (A) 40 samples with an ethanol content ≤ 4.5% v/v (LC; low alcohol content; designated as No. 1–40; Table 2) and (B) 34 samples with an ethanol content > 4.5% v/v (HC; high alcohol content; designated as No. 41–74; Table 3). The purpose of dividing into the latter mentioned group led in the diversification of samples in low alcoholic ciders (also including the alcohol-free cider samples) and high alcoholic ciders. The alcohol limit of 4.5% v/v was chosen based on real samples obtained and the plan to obtain similar number of samples in each group. For the determination of the cider BA content 16 samples were analyzed for each purchased cider (4 different manufacture batches × 4 samples per batch = 16). Additionally, 2 samples from each batch were analyzed immediately after purchase (B; at the beginning of the storage period) and 2 samples from the same batch were stored at 20 ± 2 °C (in a controlled temperature room 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). However, the number of days for which the samples were stored varied according to the particular cider samples and their best-before date (140–154 days; Tables 2 and 3).

2.3. Basic chemical analyses of cider samples

Ethanol content was assessed by Near Infrared Spectrometry using the Anton Paar Density Meter DMA 4500 M with Alcozyler Beer/Cider ME module (Anton Paar GmbH, Austria). The cider samples prior to the analyses were degassed and then filtered on laboratory filter papers. Moreover, the pH values of the tested cider samples were determined with a glass tip electrode of a calibrated pH-meter (pHSpear, Eutech Instruments, Oakton, Malaysia). Each cider sample was measured at least six times (n = 6).

2.4. Determination of biogenic amine content

Decarbonized (using an ultrasonic bath) cider samples were diluted with perchloric acid (c = 1.2 mol·l⁻¹) in a ratio of 1:1 (v/v). High performance liquid chromatography (LabAlliance, State College, USA; Agilent Technologies, Agilent, Paolo Alto, USA) after derivatization with dansyl-chloride was used for the determination of eight BA (histamine, tyramine, phenylethylamine, tryptamine, putrescine, cadaverine, spermidine and spermine). Derivatization, chromatographic separation (column: ZORBAX Eclipse Plus C18, 50 mm × 3.0 mm, 1.8 μm, Agilent Technologies) and detection [spectrophotometrically at a wavelength (λ) of 254 nm] were performed according to the methodology previously described by Dadáková et al. (2009, p. 152) and Buňka et al. (2012). Each batch of cider was analyzed from two different containers (of the same production batch; glass bottles and aluminum cans), the samples from each container were derivatized three times, and each derivatized mixture was positioned onto the chromatographic column three times (3 derivatizations × 3 repetitions × 2 samples from the each batch × 4 batches = 72; the total number of analysis through all 74 ciders was 5328).

Because of the several steps of the sample preparation, concentration of biogenic amines in the sample was corrected based on the method of internal (1,7-diaminoheptane) according to procedure of Komprda et al. (2007). Validation process of method used include also determination of the repeatability, the recovery and the limit of detection and quantification. The repeatability of the analytical process (ex-

pressed as a relative standard deviation, RSD) was determined by injecting a mixture of the biogenic amine standards after derivatization 10 times and injecting five extracts of the selected cider sample with a low BA content, respectively. The values of RSD were 0.1–0.6% and 1.2–3.9% for the instrument and method repeatability, respectively. Recoveries were evaluated using repeatedly (five times) a real cider sample with added mixture of BA standards with the concentration level of 2 mg/l (Komprda et al., 2007). The recovery of individual BA was 89.1–97.7%. The limits of detection (LOD) and quantification (LOQ) for the individual BA were in the range 0.02–0.11 mg l⁻¹ and 0.13–0.52 mg l⁻¹, respectively. The LOD (Table 1) and LOQ (Table 1) were determined according to standard chromatography procedures (Lister, 2005; Wenzl et al., 2016) and in accordance with ISO 17025 (ISO17025, 2017).

2.5. Statistical analysis

Biogenic amines results were statistically evaluated by nonparametric Kruskal–Wallis and Mann–Whitney tests. Spearman correlation coefficients between the biogenic amines content and the values of pH and ethanol content were also applied. The statistical software Unistat® 5.5 (Unistat Ltd., London, UK) and the significance level of 0.05 were applied for the tests.

3. Results and discussion

In the years 2018–2019, a total number of 74 cider samples were obtained from the Central Europe region and the content of 8 BA was determined; immediately after purchasing the sample in the retail network and at the end of the best before date. For sample comparison the ciders were divided into alcohol-free and low-alcohol (within the range of 0.21–4.48% v/v; LC) and high-alcohol (in the interval of 4.53–7.62% v/v; HC). The results of the BA content of the individual ciders are presented in Table 2 (LC) and Table 3 (HC).

Immediately after purchase, TRM was not detected in LC or HC cider samples. Nevertheless, at the end of the best before date, only 2 LC samples had a TRM concentration <5 mg l⁻¹ (p < 0.05). In the other cases, TRM was not detected at all. Moreover, TRM was one of the least detected BA in this study, a result corresponding to the findings of previously performed studies (Garai-Ibabe et al., 2013; Ladero et al., 2011). In all tested ciders (regardless to their declared ethanol content) low levels of SPD and SPN were monitored. In particular, over 90% of the tested HC and over 80% of the LC samples showed SPD and SPN concentrations below 5 mg l⁻¹ immediately after purchase and at the end of the best before date. The remaining cider samples contained 5–10 mg l⁻¹ of SPD and SPN. In general, the detected concentrations of SPD and SPN can be characterized as low. Their biosynthesis (higher polyamines) proceeds with complex pathways starting from putrescine (released from ornithine or agmatine) (Barbieri et al., 2019). Therefore, low concentrations of SPD and SPN appear regularly not only in ciders,

but also in other fermented alcoholic beverages (Anli et al., 2006; Buňka et al., 2012). In 98% of LC cider samples, PHE was not detected at the beginning of the storage period. However, only one LC sample contained PHE <5 mg l⁻¹. In the case of HC ciders, the proportion of samples above the detection limit of PHE was much higher (p < 0.05), particularly 62% of the examined HC samples showed a PHE content <5 mg l⁻¹ and in 8% of the samples the PHE content was in the range of 5 up to 10 mg l⁻¹. At the end of the best before date, the PHE content of the monitored LC samples increased (p < 0.05). Hence, 15% of the LC samples had a PHE level <5 mg l⁻¹ and 5% of the ciders showed a PHE content in the range of 5–10 mg l⁻¹. In the case of HC ciders, there were no significant changes and only one examined sample (sample No. 69) moved from the category "<5 mg l⁻¹" to "5–10 mg l⁻¹" (p < 0.05). Similar to the above mentioned BA, PHE was a BA with low detected concentrations, which corresponds to results of previously performed studies (Alvarez & Moreno-Arribas, 2014; Garai et al., 2006; Garai-Ibabe et al., 2013; Ladero et al., 2011). The detected levels of PHE, TRM, SPD and SPN can be assessed as low from the food safety point of view leading to the statement that cider does not pose significant health risks to consumers due to the occurrence of the above-mentioned BA.

Immediately after purchase, in 90% of the LC and in 62% of the HC samples no HIM was detected at all. Moreover, 8% of the LC and 35% of the HC ciders had a HIM content <5 mg l⁻¹ and 1 sample in both LC and HC groups showed a HIM concentration in the range of 5–10 mg l⁻¹ (samples No. 15 and 43). At the end of the best before date, the HIM content of the tested samples significantly increased (p < 0.05). Additionally, 8% of the tested LC samples and 24% of the HC samples reported a HIM concentration in the interval of 5–10 mg l⁻¹ and one HC cider showed a concentration of 18.1 ± 0.3 mg l⁻¹ (sample No. 52). Furthermore, similar HIM concentrations were previously reported by Garai et al. (2006) and Ladero et al. (2011). The detected amount of HIM can be assessed as low and does not pose a significant health risk to consumers. However, excessive daily intake of ciders with an HIM >10 mg l⁻¹, combined with higher ethanol concentrations, could cause health problems to the consumers (Ladero et al., 2010).

Furthermore, one of the most frequently detected BA was TYM. At the beginning of storage period (immediately after purchase), TYM was not detected in 15% of the LC and 26% of the HC samples, respectively. In addition, 68% of the LC and 41% of the HC samples reported TYM concentrations below 5 mg l⁻¹. However, 13% of the LC ciders had TYM concentrations higher than 10 mg l⁻¹. The remaining cider samples had a TYM content in the range of 5–10 mg l⁻¹. With the progress of the storage period, an increase in the TYM content in the monitored ciders was observed (p < 0.05). More than one third of the LC and HC ciders showed TYM levels <5 mg l⁻¹. Almost another one third of the LC and HC samples contained TYM in the range of 5–10 mg l⁻¹. A total number of 11 LC ciders and 2 HC products had TYM concentration in the range of 10–20 mg l⁻¹. Nevertheless, in 6 samples the TYM concentration values exceeded 20 mg l⁻¹ and ranged from 29.2 to 47.5 mg l⁻¹ (samples No. 27; 36; 43; 52; 54 and 71). The higher incidence and higher concentrations of TYM found in ciders in this study correspond to the results published by Ladero et al. (2011). The above-mentioned amounts of TYM, especially in combination with alcohol content, can cause health problems even in a healthy person (Shalaby, 1996; Silla Santos, 1996). In general, ethanol can act as HIM enhancer because it can inhibit diamino oxidases, enzymes responsible for HIM degradation. Differently, antidepressant drugs are monoamine oxidase inhibitors, enzymes involved in TYM detoxification. Moreover, according to del Rio et al. (2017) TYM and HIM can show a synergistic toxicity effect (del Rio et al., 2017).

In general, the occurrence of TYM represents a health hazard that is necessary to be taken in consideration during the construction of a system of hazard analysis and critical control points (HACCP).

Table 1

Limits of detection (LOQ) and limits of quantification for 8 biogenic amines monitored in cider samples produced in the Central Europe region.

Biogenic amine	LOD ^a (mg·l ⁻¹)	LOQ ^a (mg·l ⁻¹)
Tryptamine	0.131 ± 0.001	0.52 ± 0.02
Phenylalanine	0.063 ± 0.001	0.20 ± 0.01
Putrescine	0.110 ± 0.001	0.32 ± 0.02
Cadaverine	0.091 ± 0.001	0.26 ± 0.01
Histamine	0.110 ± 0.001	0.24 ± 0.01
Tyramine	0.012 ± 0.001	0.13 ± 0.01
Spermidine	0.021 ± 0.001	0.19 ± 0.01
Spermine	0.011 ± 0.001	0.14 ± 0.01

^a Results are expressed as mean ± standard deviation (n = 6).

Table 2

Contents of biogenic amines (mg l⁻¹) and pH-values in individual cider samples with low alcohol content ($\leq 4.5\%$ v/v^a) produced in the Central Europe region.

Number of sample	Storage period (days)	Time ^b	pH-value***	Biogenic amine content (mg l ⁻¹)****								
				Histamine	Tyramine	Putrescine	Ca daverine	Tryptamine	Phenyl-ethylamine	Spermidine	Spermine	
1	151	B	3.35 ± 0.01 ^a	ND	1.1 ± 0.1 ^a	ND	ND	ND	ND	ND	1.8 ± 0.2 ^a	2.8 ± 0.2 ^a
		E	3.16 ± 0.02 ^b	ND	4.1 ± 0.4 ^b	ND	ND	ND	ND	ND	4.5 ± 0.4 ^b	4.2 ± 0.4 ^b
2	146	B	3.54 ± 0.03 ^a	ND ^a	5.9 ± 0.6 ^a	1.9 ± 0.2 ^a	1.1 ± 0.1 ^a	ND	ND	ND	0.7 ± 0.1 ^a	3.1 ± 0.3 ^a
		E	3.38 ± 0.03 ^b	2.7 ± 0.3 ^b	14.3 ± 1.3 ^b	4.1 ± 0.3 ^b	2.8 ± 0.2 ^b	ND	ND	ND	3.7 ± 0.3 ^b	3.5 ± 0.3 ^a
3	149	B	3.11 ± 0.02 ^a	ND	ND _a	ND	ND	ND	ND	ND ^a	1.4 ± 0.1 ^a	2.4 ± 0.2 ^a
		E	2.91 ± 0.03 ^b	ND	ND _a	ND	ND	ND	ND	3.0 ± 0.3 ^b	3.0 ± 0.3 ^b	3.0 ± 0.2 ^a
4	140	B	4.51 ± 0.02 ^a	ND ^a	2.1 ± 0.2 ^a	6.6 ± 0.6 ^a	ND ^a	ND	ND	ND	3.4 ± 0.3 ^a	3.1 ± 0.3 ^a
		E	3.90 ± 0.02 ^b	4.3 ± 0.4 ^b	6.5 ± 0.6 ^b	8.7 ± 0.8 ^b	2.1 ± 0.2 ^b	ND	ND	ND	2.2 ± 0.2 ^b	3.8 ± 0.2 ^b
5	144	B	3.92 ± 0.01 ^a	ND ^a	11.7 ± 0.9 ^a	11.7 ± 1.0 ^a	19.9 ± 1.6 ^a	ND	ND	3.8 ± 0.3 ^a	2.2 ± 0.2 ^a	1.6 ± 0.2 ^a
		E	3.87 ± 0.02 ^b	2.7 ± 0.2 ^b	19.5 ± 1.8 ^b	23.5 ± 2.0 ^b	37.1 ± 3.4 ^b	ND	ND	7.7 ± 0.6 ^b	2.6 ± 0.3 ^a	2.0 ± 0.2 ^b
6	148	B	3.07 ± 0.02 ^a	ND	4.6 ± 0.4 ^a	2.6 ± 0.2 ^a	1.6 ± 0.1 ^a	ND	ND	ND	2.4 ± 0.2 ^a	8.3 ± 0.8 ^a
		E	2.73 ± 0.02 ^b	ND	15.8 ± 1.5 ^b	6.4 ± 0.6 ^b	2.4 ± 0.2 ^b	ND	ND	ND	4.8 ± 0.5 ^b	8.5 ± 0.7 ^a
7	145	B	3.11 ± 0.02 ^a	ND	ND	ND	ND	ND	ND	ND	5.8 ± 0.5 ^a	3.2 ± 0.3 ^a
		E	2.84 ± 0.02 ^b	ND	ND	ND	ND	ND	ND	ND	6.4 ± 0.5 ^a	3.8 ± 0.3 ^b
8	150	B	3.20 ± 0.02 ^a	ND	2.8 ± 0.3 ^a	ND	ND	ND	ND	ND	1.9 ± 0.2 ^a	7.2 ± 0.7 ^a
		E	2.95 ± 0.03 ^b	ND	4.0 ± 0.3 ^b	ND	ND	ND	ND	ND	3.2 ± 0.3 ^b	7.8 ± 0.6 ^a
9	145	B	5.11 ± 0.02 ^a	ND	1.4 ± 0.1 ^a	ND	ND	ND	ND	ND ^a	2.9 ± 0.2 ^a	8.2 ± 0.8 ^a
		E	4.14 ± 0.02 ^b	ND	9.2 ± 0.9 ^b	ND	2.4 ± 0.2 ^b	ND	ND	3.8 ± 0.3 ^b	2.9 ± 0.3 ^a	9.9 ± 0.8 ^b
10	146	B	3.40 ± 0.01 ^a	ND	ND ^a	ND	ND	ND	ND	ND	1.4 ± 0.1 ^a	1.4 ± 0.2 ^a
		E	3.19 ± 0.02 ^b	ND	5.3 ± 0.5 ^b	ND	ND	ND	ND	ND	4.2 ± 0.4 ^b	3.1 ± 0.3 ^b
11	149	B	3.93 ± 0.02 ^a	ND ^a	4.5 ± 0.5 ^a	22.9 ± 2.4 ^a	4.9 ± 0.5 ^a	ND	ND	ND ^a	2.4 ± 0.2 ^a	1.9 ± 0.2 ^a
		E	3.65 ± 0.02 ^b	2.3 ± 0.2 ^b	17.0 ± 1.7 ^b	31.3 ± 2.8 ^b	13.6 ± 1.0 ^b	ND	ND	6.7 ± 0.6 ^b	3.0 ± 0.3 ^b	2.7 ± 0.2 ^b
12	141	B	3.86 ± 0.02 ^a	ND	1.0 ± 0.1 ^a	ND	ND	ND	ND	ND	1.6 ± 0.2 ^a	1.8 ± 0.1 ^a
		E	3.43 ± 0.02 ^b	ND	3.6 ± 0.3 ^b	ND	ND	ND	ND	ND	1.8 ± 0.1 ^a	1.7 ± 0.2 ^b
13	149	B	3.90 ± 0.01 ^a	ND	1.9 ± 0.2 ^a	ND ^a	ND ^a	ND ^a	ND	ND	2.5 ± 0.2 ^a	2.0 ± 0.2 ^a
		E	3.25 ± 0.02 ^b	ND	8.9 ± 0.8 ^b	4.0 ± 0.4 ^b	0.9 ± 0.1 ^b	0.8 ± 0.1 ^b	ND	ND	3.8 ± 0.3 ^b	3.0 ± 0.3 ^b
14	146	B	3.15 ± 0.02 ^a	ND	1.1 ± 0.1 ^a	0.4 ± 0.1 ^a	ND	ND	ND	ND	4.9 ± 0.5 ^a	1.7 ± 0.1 ^a
		E	2.93 ± 0.02 ^b	ND	9.5 ± 0.8 ^b	3.5 ± 0.3 ^b	ND	ND	ND	ND	7.6 ± 0.7 ^b	2.7 ± 0.3 ^b
15	145	B	3.07 ± 0.01 ^a	6.7 ± 0.6 ^a	12.0 ± 1.0 ^a	14.4 ± 1.3 ^a	31.4 ± 2.8 ^a	ND	ND	ND	0.9 ± 0.1 ^a	2.9 ± 0.3 ^a
		E	2.86 ± 0.02 ^b	9.3 ± 0.8 ^b	17.8 ± 1.6 ^b	21.4 ± 2.0 ^b	42.9 ± 3.7 ^b	ND	ND	ND	1.8 ± 0.2 ^b	3.2 ± 0.3 ^a
16	149	B	3.12 ± 0.02 ^a	ND	1.7 ± 0.2 ^a	ND	ND	ND	ND	ND	1.1 ± 0.1 ^a	3.0 ± 0.3 ^a
		E	2.86 ± 0.02 ^b	ND	3.9 ± 0.3 ^b	ND	ND	ND	ND	ND	3.1 ± 0.3 ^b	3.3 ± 0.2 ^a
17	147	B	3.11 ± 0.02 ^a	ND	5.8 ± 0.6 ^a	1.1 ± 0.1 ^a	1.9 ± 0.2 ^a	ND	ND	ND	2.9 ± 0.3 ^a	3.3 ± 0.3 ^a
		E	2.43 ± 0.02 ^b	ND	17.6 ± 1.6 ^b	4.8 ± 0.4 ^b	3.0 ± 0.3 ^b	ND	ND	ND	4.2 ± 0.4 ^b	4.1 ± 0.4 ^b
18	145	B	3.18 ± 0.03 ^a	ND	ND ^a	ND	ND	ND	ND	ND	1.5 ± 0.1 ^a	6.3 ± 0.6 ^a
		E	3.01 ± 0.02 ^b	ND	5.3 ± 0.5 ^b	ND	ND	ND	ND	ND	3.8 ± 0.3 ^b	7.3 ± 0.6 ^a
19	154	B	3.14 ± 0.02 ^a	ND	0.8 ± 0.1 ^a	ND	ND	ND	ND	ND ^a	2.2 ± 0.2 ^a	3.3 ± 0.3 ^a
		E	2.95 ± 0.02 ^b	ND	2.6 ± 0.2 ^b	ND	ND	ND	ND	2.7 ± 0.2 ^b	3.0 ± 0.3 ^b	4.1 ± 0.2 ^b
20	147	B	3.52 ± 0.02 ^a	ND	1.8 ± 0.2 ^a	ND	ND	ND	ND	ND	1.9 ± 0.2 ^a	3.8 ± 0.4 ^a
		E	3.41 ± 0.01 ^b	ND	4.1 ± 0.4 ^b	ND	ND	ND	ND	ND	3.8 ± 0.3 ^b	4.0 ± 0.3 ^a
21	152	B	3.14 ± 0.02 ^a	ND	0.9 ± 0.1 ^a	ND	ND	ND	ND	ND	2.2 ± 0.2 ^a	1.1 ± 0.1 ^a
		E	2.63 ± 0.02 ^b	ND	4.0 ± 0.3 ^b	ND	ND	ND	ND	ND	3.8 ± 0.3 ^b	1.5 ± 0.1 ^b
22	149	B	3.19 ± 0.02 ^a	ND	0.6 ± 0.1 ^a	ND	ND	ND	ND	ND ^a	2.5 ± 0.3 ^a	1.8 ± 0.2 ^a
		E	2.67 ± 0.02 ^b	ND	3.3 ± 0.3 ^b	ND	ND	ND	ND	2.8 ± 0.3 ^b	4.1 ± 0.4 ^b	2.5 ± 0.2 ^b
23	150	B	3.35 ± 0.02 ^a	ND	2.5 ± 0.2 ^a	ND ^a	4.1 ± 0.3 ^a	ND	ND	ND	1.9 ± 0.2 ^a	3.6 ± 0.4 ^a
		E	2.93 ± 0.02 ^b	ND	13.7 ± 1.2 ^b	4.1 ± 0.3 ^b	6.1 ± 0.5 ^b	ND	ND	ND	2.9 ± 0.3 ^b	4.0 ± 0.2 ^a
24	141	B	3.28 ± 0.03 ^a	ND	0.9 ± 0.1 ^a	ND	ND	ND	ND	ND	2.1 ± 0.2 ^a	4.0 ± 0.3 ^a
		E	2.87 ± 0.02 ^b	ND	3.1 ± 0.3 ^b	ND	ND	ND	ND	ND	2.6 ± 0.2 ^b	4.0 ± 0.4 ^a
25	141	B	3.16 ± 0.01 ^a	ND	1.3 ± 0.1 ^a	ND ^a	ND	ND	ND	ND	1.2 ± 0.1 ^a	2.3 ± 0.2 ^a
		E	2.97 ± 0.02 ^b	ND	8.5 ± 0.7 ^b	3.2 ± 0.3 ^b	ND	ND	ND	ND	2.6 ± 0.2 ^b	3.4 ± 0.1 ^b
26	148	B	3.04 ± 0.02 ^a	ND	1.0 ± 0.1 ^a	ND	ND	ND	ND	ND	1.7 ± 0.1 ^a	1.3 ± 0.1 ^a
		E	2.90 ± 0.01 ^b	ND	3.8 ± 0.3 ^b	ND	ND	ND	ND	ND	4.6 ± 0.4 ^b	2.9 ± 0.1 ^b
27	154	B	3.34 ± 0.02 ^a	1.7 ± 0.2 ^a	12.0 ± 1.0 ^a	ND ^a	ND ^a	ND	ND	ND	2.1 ± 0.2 ^a	6.1 ± 0.6 ^a
		E	2.97 ± 0.02 ^b	7.7 ± 0.7 ^b	47.5 ± 4.1 ^b	3.7 ± 0.3 ^b	3.4 ± 0.3 ^b	ND	ND	ND	3.2 ± 0.3 ^b	7.5 ± 0.7 ^a
28	153	B	3.63 ± 0.01 ^a	ND	1.4 ± 0.1 ^a	ND	ND	ND	ND	ND	2.2 ± 0.2 ^a	1.4 ± 0.1 ^a
		E	2.92 ± 0.02 ^b	ND	2.9 ± 0.3 ^b	ND	ND	ND	ND	ND	3.1 ± 0.3 ^b	1.5 ± 0.1 ^a
29	147	B	4.55 ± 0.01 ^a	0.9 ± 0.1 ^a	12.9 ± 1.2 ^a	15.8 ± 1.3 ^a	ND	ND	ND	ND	2.3 ± 0.1 ^a	2.3 ± 0.2 ^a
		E	3.90 ± 0.02 ^b	3.4 ± 0.3 ^b	18.7 ± 1.8 ^b	45.9 ± 3.9 ^b	ND	ND	ND	ND	2.4 ± 0.2 ^a	4.3 ± 0.4 ^b
30	142	B	3.79 ± 0.02 ^a	ND	2.2 ± 0.2 ^a	ND	1.7 ± 0.2 ^a	ND	ND	ND	2.4 ± 0.2 ^a	2.8 ± 0.2 ^a
		E	2.72 ± 0.02 ^b	ND	9.0 ± 0.8 ^b	ND	2.9 ± 0.3 ^b	ND	ND	ND	4.4 ± 0.4 ^b	3.6 ± 0.4 ^b
31	146	B	3.39 ± 0.02 ^a	ND	3.9 ± 0.4 ^a	ND	ND	ND	ND	ND	2.3 ± 0.2 ^a	5.9 ± 0.5 ^a
		E	3.34 ± 0.02 ^a	ND	4.5 ± 0.4 ^b	ND	ND	ND	ND	ND	3.6 ± 0.4 ^b	8.2 ± 0.7 ^b
32	142	B	3.01 ± 0.01 ^a	ND	3.5 ± 0.3 ^a	ND ^a	ND	ND	ND	ND ^a	0.9 ± 0.1 ^a	2.1 ± 0.2 ^a
		E	3.02 ± 0.02 ^a	ND	13.7 ± 1.2 ^b	2.0 ± 0.2 ^b	ND	ND	ND	2.5 ± 0.3 ^b	1.5 ± 0.2 ^b	2.5 ± 0.2 ^b
33	148	B	2.69 ± 0.01 ^a	ND	3.9 ± 0.3 ^a	2.5 ± 0.2 ^a	ND ^a	ND	ND	ND	2.7 ± 0.2 ^a	3.9 ± 0.4 ^a

(continued on next page)

Table 2 (continued)

Number of sample	Storage period (days)	Time ^b	pH-value ^{***}	Biogenic amine content (mg l ⁻¹) ^{****}							
				Histamine	Tyramine	Putrescine	Ca daverine	Tryptamine	Phenyl-ethylamine	Spermidine	Spermine
34	149	E	2.61 ± 0.02 ^b	ND	17.0 ± 1.5 ^b	6.0 ± 0.6 ^b	1.9 ± 0.2 ^b	ND	ND	2.8 ± 0.2 ^a	4.2 ± 0.4 ^b
		B	2.69 ± 0.02 ^a	ND	ND ^a	ND	ND	ND	ND	1.6 ± 0.1 ^a	3.4 ± 0.3 ^a
35	142	E	2.70 ± 0.01 ^a	ND	8.8 ± 0.8 ^b	ND	ND	ND	ND	3.3 ± 0.3 ^b	4.7 ± 0.4 ^b
		B	3.00 ± 0.03 ^a	ND	1.4 ± 0.1 ^a	ND	ND	ND	ND	2.7 ± 0.3 ^a	7.6 ± 0.6 ^a
36	151	E	2.90 ± 0.01 ^b	ND	6.9 ± 0.6 ^b	ND	ND	ND	ND	4.9 ± 0.4 ^b	8.5 ± 0.8 ^a
		B	2.63 ± 0.03 ^a	2.1 ± 0.2 ^a	15.1 ± 1.1 ^a	7.5 ± 0.7 ^a	26.9 ± 2.5 ^a	ND	ND	2.8 ± 0.2 ^a	2.5 ± 0.2 ^a
37	153	E	2.59 ± 0.03 ^a	8.9 ± 0.8 ^b	39.7 ± 3.5 ^b	18.3 ± 1.6 ^b	40.4 ± 3.4 ^b	ND	ND	2.8 ± 0.1 ^a	3.2 ± 0.3 ^b
		B	3.85 ± 0.02 ^a	ND	2.2 ± 0.2 ^a	ND	1.5 ± 0.1 ^a	ND ^a	ND	1.7 ± 0.2 ^a	2.6 ± 0.3 ^a
38	146	E	3.54 ± 0.03 ^b	ND	9.2 ± 0.8 ^b	ND	3.9 ± 0.3 ^b	1.1 ± 0.1 ^b	ND	3.5 ± 0.3 ^b	3.3 ± 0.3 ^b
		B	4.29 ± 0.02 ^a	ND	2.6 ± 0.2 ^a	ND	ND	ND	ND	1.7 ± 0.2 ^a	1.9 ± 0.2 ^a
39	144	E	3.50 ± 0.02 ^b	ND	6.9 ± 0.6 ^b	ND	ND	ND	ND	3.5 ± 0.3 ^b	2.3 ± 0.2 ^b
		B	4.39 ± 0.02 ^a	ND ^a	4.8 ± 0.4 ^a	9.4 ± 0.9 ^a	4.5 ± 0.4 ^a	ND	ND	2.0 ± 0.1 ^a	1.9 ± 0.2 ^a
40	141	E	3.60 ± 0.02 ^b	3.9 ± 0.3 ^b	16.5 ± 1.6 ^b	12.0 ± 1.1 ^b	18.1 ± 1.7 ^b	ND	ND	3.9 ± 0.4 ^b	2.6 ± 0.2 ^b
		B	4.95 ± 0.02 ^a	ND	ND ^a	ND	ND	ND	ND ^a	2.2 ± 0.2 ^a	2.6 ± 0.2 ^a
		E	3.90 ± 0.02 ^b	ND	3.2 ± 0.3 ^b	ND	ND	ND	1.3 ± 0.1 ^b	4.3 ± 0.4 ^b	2.9 ± 0.2 ^a

*** The means within a column (the difference between pH-values immediately after purchase and at the end of best before date) followed by different superscript letters differ ($p < 0.05$); each sample was evaluated separately.

**** The means within a column (the difference between BA amount immediately after purchase and at the end of best before date) followed by different superscript letters differ ($p < 0.05$); each sample was evaluated separately.

^a Ethanol content was assessed by Near Infrared Spectrometry and confirmed by the values declared on the bottle vignettes.

^b Time of sampling: B – at the beginning of storage (immediately after purchase); E – at the end of storage (at the end of best before date).

Moreover, a significant content of PUT and CAD was also detected in the monitored cider samples. Immediately after purchase, no PUT was detected in 70% of the LC products, however, 13% of the ciders contained PUT < 5 mg l⁻¹ and 16% of samples showed a PUT level; in the range of 5–20 mg l⁻¹. At the beginning of the storage period, the PUT content of one LC sample was found to be 22.9 ± 0.9 mg l⁻¹ (sample No. 11). On the whole, in the case of HC ciders, PUT was not detected in only 1 sample. Almost 80% of the tested products had a PUT level < 5 mg l⁻¹, whilst 12% of the samples within the range of 5–20 mg l⁻¹. Furthermore, immediately after purchase two HC samples reported a PUT concentration of 25.6 ± 1.0 mg l⁻¹ (sample No. 43) and 32.4 ± 1.8 mg l⁻¹ (sample No. 52), respectively. In more than 70% of the LC ciders and more than 50% of the HC samples, CAD was not detected immediately after purchase. Additionally, in 20% of the LC products and in more than 26% of the HC samples, the CAD content was found to be < 5 mg l⁻¹. Moreover, in 3% of the LC and in 21% of the HC ciders the CAD content was detected to be in the range of 5–20 mg l⁻¹. In two LC ciders, the CAD content of 26.0 ± 0.9 mg l⁻¹ (sample No. 36) and 31.4 ± 1.1 mg l⁻¹ (sample No. 15), respectively, was found at the beginning of the storage period. Over the storage period, a significant increase in the content of PUT and CAD ($p < 0.05$; Tables 2 and 3) was observed. In 58% of the tested LC ciders, PUT was not detected at the end of the best before date ($p < 0.05$). On the other hand, in the case of HC ciders, PUT was detected in all examined products. In particular, PUT content < 5 mg l⁻¹ was detected in 20% of the LC and in 47% of the HC samples, respectively. In 13% of the LC ciders and in more than 40% of the ciders characterized as HC, the PUT content was in the range of 5–20 mg l⁻¹. Moreover, in four LC products (samples No. 5; 11; 15 and 29) and in four HC samples (samples No. 43; 52; 62 and 69) the PUT content reached values in the range of 20.4–45.9 mg l⁻¹. Furthermore, CAD was not detected in 60% of the LC and in 24% of the HC ciders at the end of the storage period. The CAD content was < 5 mg l⁻¹ in 25% of the LC samples and in more than 50% of the HC ciders. Three LC products and eight HC products reported CAD concentrations in the interval of 5–20 mg l⁻¹. At the end of the best before date, three LC samples had a CAD content in the range of 37.1–42.9 mg l⁻¹ (samples No. 5; 15 and 36). The results are in agreement to those of Ladero et al. (2011). The concentrations of PUT and CAD reported in this study are not hazardous to a healthy consumer. On the other hand, PUT and CAD can potentiate the effects of HIM and

TYM and thus, the occurrence of PUT and CAD together with HIM and TYM can cause health problems even in healthy individuals (Alvarez & Moreno-Arribas, 2014). Therefore, it is necessary to monitor and evaluate the content of these BA from the perspective of the HACCP system.

In general, the formation of BA in fermented alcoholic beverages (including cider, beer and wine) is affected by several factors, such as present microorganisms, pH, ethanol content, sulfur anhydrite content, fermentation process, quality of the applied raw materials and processing parameters. Furthermore, the presence of indigenous heterofermentative LAB (mainly representatives of the genera of *Lactobacillus*, *Oenococcus*, *Pediococcus*) could be the prevailing reason for promoting the development of BA in cider (Cousin et al., 2017; Lorencová et al., 2020).

In Fig. 1 (part C) is depicted the total content of the sum of the eight monitored BA in all 74 tested ciders from the region of Central Europe. No sample at the end of the best before date had a sum of BA below < 5 mg l⁻¹, which can be considered practically as safe even for alcoholic beverages. Similarly, for healthy individuals, another 67% of the samples can be evaluated as safe at the beginning of the storage period and 47% of the cider samples after the best before date, where the total BA content was in the range of 5–20 mg l⁻¹. Moreover, a total number of 14% of the samples immediately after purchase and 31% of samples at the end of the best before date showed a BA concentration in the range of 20–50 mg l⁻¹. The latter finding can be considered as hazardous for some consumers (e.g. using medicaments inhibiting the action of the detoxification system). Nevertheless, 16 samples (22%) were found to have a BA content above 50 mg l⁻¹ at the end of the best before date, which may have a dangerous effect to a healthy consumer in combination with ethanol (Tofalo et al., 2016, p. 424). In addition, one LC cider (sample No. 36) and two HC products (samples No. 43 and 53) showed a sum of BA almost 120 mg l⁻¹. In general, the total mean BA contents in cider samples were lower than values reported previously by various authors in wines and beers (Angulo et al., 2020; Lorencová et al., 2020; Palomino-Vasco et al., 2019).

According to the results presented in Fig. 1 (part A and B), it could be reported that the distribution of total BA content “moves” to the categories “10–20”, “20–50” and “> 50” at the end of the storage time. In general, the content of BA increased with the progress of storage time, regardless the samples ethanol content. Furthermore, the LC samples presented higher values of BA compared to HC samples. A possible

Table 3

Contents of biogenic amines (mg·l⁻¹) and pH-values in individual cider samples with high alcohol content ($\leq 4.5\%$ v/v^a) produced in the Central Europe region.

Number of sample	Storage period (days)	Time ^b	pH-value ^{***}	Biogenic amine content (mg·l ⁻¹) ^{****}							
				Histamine	Tyramine	Putrescine	Cadaverine	Tryptamine	Phenylethylamine	Spermidine	Spermine
41	142	B	4.52 ± 0.01 ^a	ND	5.8 ± 0.5 ^a	1.9 ± 0.2 ^a	1.4 ± 0.1 ^a	ND	2.0 ± 0.2 ^a	2.3 ± 0.2 ^a	6.5 ± 0.6 ^a
		E	3.38 ± 0.02 ^b	ND	9.4 ± 0.9 ^b	5.4 ± 0.5 ^b	3.0 ± 0.2 ^b	ND	2.9 ± 0.2 ^b	3.4 ± 0.3 ^b	7.5 ± 0.7 ^a
42	158	B	4.55 ± 0.02 ^a	ND	1.7 ± 0.2 ^a	1.4 ± 0.1 ^a	ND ^a	ND	ND	1.4 ± 0.1 ^a	3.1 ± 0.3 ^a
		E	3.35 ± 0.03 ^b	ND	4.1 ± 0.3 ^b	3.5 ± 0.4 ^b	1.8 ± 0.2 ^b	ND	ND	3.0 ± 0.3 ^b	3.5 ± 0.3 ^a
43	146	B	4.60 ± 0.02 ^a	5.8 ± 0.5 ^a	8.3 ± 0.7 ^a	25.6 ± 2.3 ^a	14.9 ± 1.2 ^a	ND	1.5 ± 0.1 ^a	1.7 ± 0.2 ^a	2.2 ± 0.2 ^a
		E	3.50 ± 0.02 ^b	9.2 ± 0.8 ^b	41.0 ± 3.7 ^b	37.4 ± 3.3 ^b	19.6 ± 1.8 ^b	ND	3.1 ± 0.3 ^b	2.8 ± 0.2 ^b	3.9 ± 0.3 ^b
44	148	B	3.43 ± 0.02 ^a	ND	ND	3.6 ± 0.3 ^a	ND ^a	ND	ND	2.7 ± 0.2 ^a	2.8 ± 0.2 ^a
		E	3.16 ± 0.01 ^b	ND	ND	8.2 ± 0.8 ^b	2.0 ± 0.2 ^b	ND	ND	3.6 ± 0.3 ^b	3.3 ± 0.3 ^b
45	144	B	3.79 ± 0.02 ^a	ND ^a	1.0 ± 0.1 ^a	2.1 ± 0.2 ^a	1.0 ± 0.1 ^a	ND	ND	2.2 ± 0.2 ^a	3.0 ± 0.3 ^a
		E	3.51 ± 0.02 ^b	1.7 ± 0.2 ^b	2.5 ± 0.2 ^b	2.4 ± 0.2 ^b	2.3 ± 0.2 ^b	ND	ND	2.8 ± 0.2 ^b	4.2 ± 0.4 ^b
46	140	B	3.97 ± 0.02 ^a	ND ^a	2.9 ± 0.3 ^a	1.2 ± 0.1 ^a	ND ^a	ND	0.7 ± 0.1 ^a	1.6 ± 0.1 ^a	3.0 ± 0.3 ^a
		E	3.91 ± 0.02 ^b	4.5 ± 0.3 ^b	7.2 ± 0.7 ^b	6.1 ± 0.5 ^b	2.8 ± 0.3 ^b	ND	1.0 ± 0.1 ^b	2.2 ± 0.2 ^b	3.8 ± 0.3 ^b
47	142	B	3.89 ± 0.02 ^a	2.3 ± 0.2 ^a	6.7 ± 0.6 ^a	4.8 ± 0.4 ^a	5.2 ± 0.5 ^a	ND	7.5 ± 0.7 ^a	2.2 ± 0.2 ^a	2.7 ± 0.2 ^a
		E	3.62 ± 0.02 ^b	9.4 ± 0.8 ^b	15.6 ± 1.4 ^b	13.2 ± 1.2 ^b	9.6 ± 0.9 ^b	ND	8.3 ± 0.8 ^b	3.5 ± 0.3 ^b	3.9 ± 0.3 ^b
48	147	B	3.90 ± 0.02 ^a	ND	3.1 ± 0.3 ^a	2.0 ± 0.2 ^a	1.1 ± 0.1 ^a	ND	2.1 ± 0.2 ^a	2.6 ± 0.2 ^a	2.9 ± 0.3 ^a
		E	3.57 ± 0.01 ^b	ND	6.3 ± 0.6 ^b	7.7 ± 0.7 ^b	2.4 ± 0.2 ^b	ND	2.9 ± 0.3 ^b	3.6 ± 0.3 ^b	3.1 ± 0.3 ^a
49	144	B	3.92 ± 0.02 ^a	ND	2.0 ± 0.2 ^a	1.1 ± 0.1 ^a	1.1 ± 0.1 ^a	ND	1.9 ± 0.2 ^a	2.4 ± 0.2 ^a	2.8 ± 0.2 ^a
		E	3.61 ± 0.03 ^b	ND	3.5 ± 0.3 ^b	3.9 ± 0.3 ^b	2.8 ± 0.3 ^b	ND	2.2 ± 0.2 ^a	3.3 ± 0.3 ^b	3.8 ± 0.3 ^b
50	143	B	3.05 ± 0.02 ^a	ND ^a	6.5 ± 0.5 ^a	2.9 ± 0.3 ^a	ND ^a	ND	2.3 ± 0.2 ^a	2.7 ± 0.2 ^a	2.9 ± 0.3 ^a
		E	2.78 ± 0.02 ^b	2.3 ± 0.2 ^b	8.2 ± 0.7 ^b	7.0 ± 0.6 ^b	1.5 ± 0.1 ^b	ND	2.8 ± 0.3 ^b	3.7 ± 0.3 ^b	3.3 ± 0.3 ^b
51	150	B	3.94 ± 0.02 ^a	ND	ND	2.2 ± 0.2 ^a	ND	ND	2.0 ± 0.2 ^a	2.2 ± 0.2 ^a	2.4 ± 0.2 ^a
		E	3.91 ± 0.02 ^a	ND	ND	4.5 ± 0.4 ^b	ND	ND	2.9 ± 0.2 ^b	2.6 ± 0.2 ^a	2.5 ± 0.2 ^a
52	149	B	3.92 ± 0.02 ^a	4.0 ± 0.3 ^a	9.1 ± 0.8 ^a	32.4 ± 3.0 ^a	6.2 ± 0.5 ^a	ND	ND	2.9 ± 0.3 ^a	3.9 ± 0.3 ^a
		E	3.39 ± 0.01 ^b	18.1 ± 1.7 ^b	29.2 ± 2.4 ^b	45.0 ± 4.1 ^b	17.6 ± 1.6 ^b	ND	ND	3.4 ± 0.3 ^b	4.7 ± 0.3 ^b
53	152	B	3.92 ± 0.03 ^a	3.2 ± 0.3 ^a	2.1 ± 0.2 ^a	7.0 ± 0.6 ^a	5.4 ± 0.5 ^a	ND	8.9 ± 0.8 ^a	2.5 ± 0.2 ^a	2.6 ± 0.3 ^a
		E	3.91 ± 0.02 ^a	9.7 ± 1.0 ^b	7.3 ± 0.6 ^b	10.1 ± 1.0 ^b	7.1 ± 0.6 ^b	ND	9.6 ± 0.9 ^a	3.1 ± 0.2 ^b	3.5 ± 0.3 ^b
54	151	B	3.80 ± 0.02 ^a	ND ^a	6.6 ± 0.6 ^a	3.9 ± 0.4 ^a	1.3 ± 0.1 ^a	ND	2.0 ± 0.2 ^a	2.7 ± 0.2 ^a	2.4 ± 0.2 ^a
		E	3.56 ± 0.03 ^b	2.2 ± 0.2 ^b	31.8 ± 2.9 ^b	9.3 ± 0.9 ^b	2.8 ± 0.3 ^b	ND	2.6 ± 0.2 ^b	2.9 ± 0.3 ^a	3.1 ± 0.3 ^b
55	149	B	5.14 ± 0.02 ^a	0.7 ± 0.1 ^a	1.0 ± 0.1 ^a	2.5 ± 0.2 ^a	1.0 ± 0.1 ^a	ND	ND	2.3 ± 0.2 ^a	3.4 ± 0.3 ^a
		E	4.11 ± 0.02 ^b	3.4 ± 0.4 ^b	3.6 ± 0.3 ^b	4.6 ± 0.4 ^b	2.2 ± 0.2 ^a	ND	ND	2.5 ± 0.2 ^a	3.8 ± 0.3 ^a
56	144	B	3.66 ± 0.02 ^a	ND	ND	ND ^a	ND	ND	ND	2.4 ± 0.2 ^a	2.9 ± 0.3 ^a
		E	3.37 ± 0.01 ^b	ND	ND	3.0 ± 0.3 ^b	ND	ND	ND	3.2 ± 0.3 ^b	4.5 ± 0.4 ^b
57	155	B	3.88 ± 0.03 ^a	ND	2.2 ± 0.2 ^a	2.9 ± 0.3 ^a	ND ^a	ND	ND	2.5 ± 0.2 ^a	3.5 ± 0.3 ^a
		E	3.62 ± 0.02 ^b	ND	2.3 ± 0.2 ^b	8.7 ± 0.7 ^b	2.1 ± 0.2 ^a	ND	ND	2.6 ± 0.3 ^a	5.6 ± 0.5 ^b
58	148	B	3.89 ± 0.02 ^a	1.6 ± 0.1 ^a	ND ^a	1.6 ± 0.1 ^a	ND ^a	ND	2.1 ± 0.2 ^a	3.2 ± 0.3 ^a	1.7 ± 0.1 ^a
		E	3.65 ± 0.02 ^b	8.0 ± 0.8 ^b	3.0 ± 0.3 ^b	3.6 ± 0.3 ^b	2.0 ± 0.2 ^a	ND	2.7 ± 0.2 ^b	3.8 ± 0.3 ^b	3.4 ± 0.3 ^b
59	152	B	3.41 ± 0.02 ^a	2.8 ± 0.3 ^a	6.6 ± 0.6 ^a	5.7 ± 0.5 ^a	6.1 ± 0.6 ^a	ND	1.8 ± 0.2 ^a	2.2 ± 0.2 ^a	3.4 ± 0.3 ^a
		E	3.18 ± 0.02 ^b	9.8 ± 0.8 ^b	8.8 ± 0.8 ^b	19.1 ± 1.7 ^b	8.5 ± 0.7 ^a	ND	2.8 ± 0.2 ^b	3.6 ± 0.3 ^b	3.7 ± 0.2 ^a
60	154	B	3.66 ± 0.02 ^a	ND	ND ^a	2.8 ± 0.2 ^a	ND	ND	1.1 ± 0.1 ^a	3.1 ± 0.3 ^a	2.4 ± 0.2 ^a
		E	3.41 ± 0.02 ^b	ND	1.4 ± 0.1 ^b	4.5 ± 0.4 ^b	ND	ND	2.8 ± 0.3 ^b	4.3 ± 0.4 ^b	4.1 ± 0.4 ^b
61	147	B	3.66 ± 0.02 ^a	0.5 ± 0.1 ^a	1.9 ± 0.1 ^a	1.7 ± 0.1 ^a	ND ^a	ND	1.6 ± 0.2 ^a	2.6 ± 0.2 ^a	3.2 ± 0.3 ^a
		E	3.39 ± 0.02 ^b	2.5 ± 0.2 ^b	4.2 ± 0.4 ^b	3.8 ± 0.3 ^b	1.6 ± 0.1 ^a	ND	2.6 ± 0.2 ^b	2.8 ± 0.1 ^a	3.4 ± 0.3 ^a
62	145	B	4.65 ± 0.02 ^a	2.8 ± 0.3 ^a	6.0 ± 0.5 ^a	14.8 ± 1.3 ^a	8.9 ± 0.9 ^a	ND	ND	1.5 ± 0.1 ^a	3.1 ± 0.3 ^a
		E	3.18 ± 0.03 ^b	7.0 ± 0.6 ^b	9.2 ± 0.8 ^b	20.4 ± 1.9 ^b	19.9 ± 1.7 ^a	ND	ND	2.1 ± 0.2 ^b	4.6 ± 0.4 ^b
63	145	B	3.61 ± 0.02 ^a	0.9 ± 0.1 ^a	0.8 ± 0.1 ^a	1.8 ± 0.2 ^a	ND	ND	2.3 ± 0.2 ^a	2.8 ± 0.2 ^a	1.7 ± 0.2 ^a
		E	3.31 ± 0.02 ^b	3.0 ± 0.3 ^b	2.7 ± 0.3 ^b	4.3 ± 0.4 ^b	ND	ND	3.4 ± 0.3 ^b	2.9 ± 0.3 ^a	4.3 ± 0.4 ^b
64	148	B	4.03 ± 0.02 ^a	ND	ND	2.9 ± 0.3 ^a	ND	ND	ND	2.8 ± 0.2 ^a	1.6 ± 0.2 ^a
		E	3.78 ± 0.02 ^b	ND	ND	4.0 ± 0.4 ^b	ND	ND	ND	2.8 ± 0.3 ^a	1.8 ± 0.3 ^a
65	153	B	3.57 ± 0.02 ^a	ND ^a	1.3 ± 0.1 ^a	3.0 ± 0.3 ^a	1.3 ± 0.1 ^a	ND	2.7 ± 0.3 ^a	1.2 ± 0.1 ^a	1.8 ± 0.2 ^a
		E	3.30 ± 0.02 ^b	2.2 ± 0.2 ^b	9.4 ± 0.8 ^b	7.0 ± 0.7 ^b	4.0 ± 0.4 ^a	ND	3.1 ± 0.3 ^a	2.5 ± 0.2 ^b	4.8 ± 0.5 ^b
66	147	B	5.04 ± 0.02 ^a	0.6 ± 0.1 ^a	2.2 ± 0.2 ^a	2.7 ± 0.2 ^a	ND	ND	1.3 ± 0.1 ^a	2.2 ± 0.2 ^a	3.0 ± 0.3 ^a
		E	3.75 ± 0.02 ^b	2.1 ± 0.2 ^b	4.4 ± 0.4 ^b	4.4 ± 0.4 ^b	ND	ND	2.8 ± 0.3 ^b	2.6 ± 0.2 ^b	3.6 ± 0.3 ^b
67	140	B	3.98 ± 0.03 ^a	ND	0.5 ± 0.0 ^a	3.0 ± 0.3 ^a	ND ^a	ND	1.8 ± 0.2 ^a	3.3 ± 0.3 ^a	2.9 ± 0.3 ^a
		E	3.71 ± 0.01 ^b	ND	2.1 ± 0.2 ^b	4.3 ± 0.4 ^b	1.9 ± 0.2 ^a	ND	3.2 ± 0.3 ^b	3.9 ± 0.3 ^b	4.3 ± 0.2 ^a
68	150	B	3.59 ± 0.02 ^a	0.8 ± 0.1 ^a	1.7 ± 0.2 ^a	2.2 ± 0.2 ^a	ND	ND	ND	2.5 ± 0.2 ^a	2.1 ± 0.2 ^a
		E	3.35 ± 0.02 ^b	5.3 ± 0.5 ^b	2.6 ± 0.2 ^b	3.4 ± 0.3 ^b	ND	ND	ND	2.8 ± 0.2 ^a	4.0 ± 0.4 ^b
69	149	B	3.72 ± 0.02 ^a	2.8 ± 0.3 ^a	5.9 ± 0.5 ^a	16.8 ± 1.4 ^a	5.6 ± 0.5 ^a	ND	3.2 ± 0.3 ^a	3.4 ± 0.3 ^a	2.7 ± 0.2 ^a
		E	3.43 ± 0.03 ^b	7.0 ± 0.6 ^b	17.1 ± 1.6 ^b	40.4 ± 3.7 ^b	7.9 ± 0.7 ^a	ND	8.3 ± 0.7 ^b	4.8 ± 0.5 ^b	4.3 ± 0.4 ^b
70	153	B	3.90 ± 0.02 ^a	ND	ND	2.0 ± 0.1 ^a	ND	ND	2.9 ± 0.3 ^a	2.0 ± 0.2 ^a	8.8 ± 0.7 ^a
		E	3.61 ± 0.01 ^b	ND	ND	3.1 ± 0.3 ^b	ND	ND	3.5 ± 0.3 ^b	2.2 ± 0.2 ^a	9.1 ± 0.6 ^a
71	152	B	3.88 ± 0.02 ^a	ND ^a	7.9 ± 0.7 ^a	3.2 ± 0.3 ^a	2.4 ± 0.2 ^a	ND	6.1 ± 0.5 ^a	2.8 ± 0.3 ^a	3.2 ± 0.3 ^a
		E	3.65 ± 0.03 ^b	2.1 ± 0.2 ^b	47.3 ± 4.1 ^b	6.2 ± 0.6 ^b	6.7 ± 0.6 ^b	ND	7.8 ± 0.7 ^b	3.3 ± 0.2 ^a	3.4 ± 0.3 ^a
72	148	B	3.90 ± 0.01 ^a	ND	5.9 ± 0.5 ^a	1.8 ± 0.2 ^a	ND ^a	ND	1.8 ± 0.3 ^a	2.3 ± 0.2 ^a	3.6 ± 0.3 ^a
		E	3.79 ± 0.02 ^b	ND	7.3 ± 0.7 ^b	4.1 ± 0.4 ^b	0.9 ± 0.1 ^b	ND	3.4 ± 0.3 ^b	2.4 ± 0.2 ^a	3.7 ± 0.3 ^a
73	147	B	3.67 ± 0.02 ^a	ND	ND	2.4 ± 0.2 ^a	ND ^a	ND	2.3 ± 0.2 ^a	1.6 ± 0.1 ^a	2.3 ± 0.2 ^a

(continued on next page)

Table 3 (continued)

Number of sample	Storage period (days)	Time ^b	pH-value ^{***}	Biogenic amine content (mg·l ⁻¹) ^{****}							
				Histamine	Tyramine	Putrescine	Cadaverine	Tryptamine	Phenylethylamine	Spermidine	Spermine
74	151	E	3.22 ± 0.02 ^b	ND	ND	7.4 ± 0.7 ^b	2.2 ± 0.2 ^b	ND	3.2 ± 0.3 ^b	2.5 ± 0.2 ^b	4.6 ± 0.4 ^b
		B	3.89 ± 0.02 ^a	ND	ND ^a	3.7 ± 0.3 ^a	0.7 ± 0.1 ^a	ND	2.8 ± 0.3 ^a	2.9 ± 0.3 ^a	2.6 ± 0.2 ^a
		E	3.64 ± 0.02 ^b	ND	9.7 ± 0.9 ^b	8.2 ± 0.8 ^b	3.2 ± 0.3 ^b	ND	3.0 ± 0.3 ^a	3.0 ± 0.2 ^a	3.5 ± 0.3 ^b

^{***} The means within a column (the difference between pH-values immediately after purchase and at the end of best before date) followed by different superscript letters differ ($p < 0.05$); each sample was evaluated separately.

^{****} The means within a column (the difference between BA amount immediately after purchase and at the end of best before date) followed by different superscript letters differ ($p < 0.05$); each sample was evaluated separately.

^a Ethanol content was assessed by Near Infrared Spectrometry and confirmed by the values declared on the bottle vignettes.

^b Time of sampling: B – at the beginning of storage (immediately after purchase); E – at the end of storage (at the end of best before date).

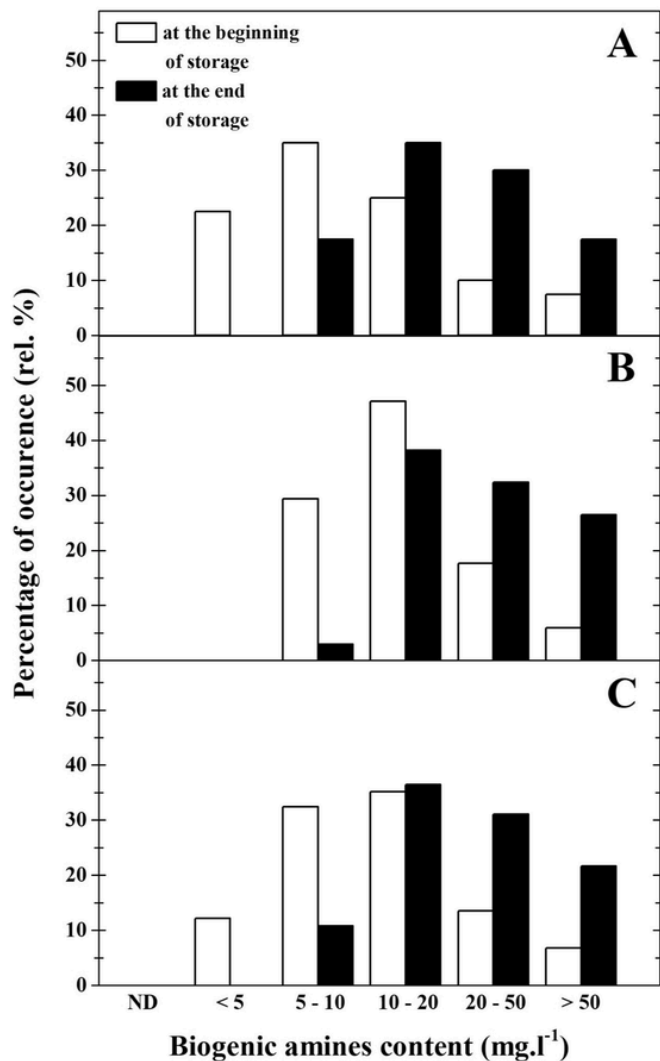


Fig. 1. The occurrence of total content of biogenic amines (mg·l⁻¹) in ciders produced in the Central Europe region of at the beginning of storage (immediately after purchase; white columns) and at the end of storage period (end of the best before date; black columns). The results are expressed as percentage of content of cider samples tested (part A – 40 samples with low alcohol content - ethanol content ≤ 4.5% v/v; part B – 34 samples with high alcohol content - ethanol content > 4.5% v/v; part C – 74 samples in total); ND = not detected.

explanation of the above-mentioned observation could be based on the presence of decarboxylase-positive microorganisms during the fermentation process of the LC samples (bib Anli and Bayram, 2008; Anli & Bayram, 2008). In addition, the environment during LC cider fermentation (low ethanol content and availability of free amino acids) is probably providing favorable conditions allowing bacterial growth and decarboxylase synthesis and activity, leading to elevated values of BA. Moreover, intensive BA accumulation can represent a cellular defense to withstand acid stress. Under acidic condition (low pH; Tables 1 and 2) the transcription of many decarboxylase genes might be induced leading to improved cell performances such formation of BA. Generally, BA are formed by decarboxylating and proteolytic enzymes that are produced by microorganisms naturally present in native microbial flora, or microorganisms added as starter culture, or added through contamination (Barbieri et al., 2019; Vinci & Maddaloni, 2020).

Although the consumption of food/beverages containing high levels of BA can have toxicological consequences, there is no specific legislation regarding the presence of BA, with the exception of fishery products, for which the maximum acceptable level of histamine is defined. In addition, upper limits for BA in other foods have only been recommended/suggested (100 mg of HIM per kg of food or 2 mg of HIM per liter of alcoholic beverage). In alcoholic beverages, the toxic dose is considered to be in the range of 8–20 mg l⁻¹ for HIM, in the range of 25–40 mg l⁻¹ for TYM, whereas as little as 3 mg l⁻¹ of PHE could cause negative physiological effects (Barbieri et al., 2019; Spano et al., 2010). Therefore, from the obtained results (Tables 2 and 3) it could be concluded that 85% of the tested samples were safe, regarding HIM suggested limits. In the case of TYM suggested limits, 75% of the ciders could be labelled as safe.

Nevertheless, as a response to the above-mentioned unfavorable facts, stricter compliance with hygiene standards for cider production and distribution and a review of the HACCP system could be proposed. The simultaneous effects of the BA and also alcohol occurrence should be also take into account. This products could be hazardous for sensitive consumers. In addition, the knowledge of BA profile in cider is of great importance for both consumers and producers. Monitoring of BA profile (types and concentration) in ciders could serve as a fingerprint providing valuable information for safety and quality control during the manufacture, distribution and storage of cider.

In addition, the LC cider samples presented pH values in the range of 3.01–5.11 at the beginning of the storage period and at the end of the storage period within the range of 2.61–3.90. Moreover, in the case of HC samples the initial pH of the samples was within the range of 3.05–5.04 and at the end of the storage period was in the range of 2.78–3.91. From the results it could be stated that the pH of all samples (regardless of the ethanol content) decreased with the progress of storage time. The latter decrease in the pH values could be a result of microorganism (LAB and/or yeast) action, probably due to insufficient thermal treatment of the ciders. According to the results obtained by correlation analysis the p-values for the correlation between the con-

tent of 8 BA and the ethanol content and between the content of 8 BA and the pH values were both higher than the significance level 0.05 ($p \geq 0.05$). The above-mentioned results indicate that there is no inclusive evidence about the significance of the association between the variables.

4. Conclusions

The most abundant monitored BA in the tested cider samples were TYM, PUT and CAD. On the contrary, low concentrations of TRM, SPD, SPN, HIM and PHE were reported. In general, the prolonging of the storage period resulted in elevated levels of BA (regardless of the ethanol content of the tested ciders). Furthermore, immediately after purchase a total number of 14% of the samples and at the end of the best before date 31% of the samples showed a BA concentration within the interval of 20–50 mg l⁻¹. Nevertheless, the BA content of 16 samples was higher than 50 mg l⁻¹ at the end of the best before date. However, one LC sample and two HC products showed a sum of BA nearly 120 mg l⁻¹. Additionally, the LC samples presented higher values of BA compared to HC samples. In particular, higher concentrations of TYM, CAD and PUT were detected in LC samples. From the obtained results it could be concluded that 85% of the tested samples were safe, regarding HIM suggested limits. However, in the case of TYM, 75% of the ciders could be labelled as safe. The origin of the BA detected in cider samples could be a result from the BA already present in the utilized raw materials or be synthesized during the production process. However, the BA profile detected in the tested cider samples during storage suggest that BA are synthesized and accumulated with the prolonging of the storage period. We could conclude that stricter compliance with hygiene standards for cider production and distribution and a review of the HACCP system could be introduced.

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