DETERMINATION OF SELECTED BIOGENIC AMINES IN FERMENTED VEGETABLES JUICES

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Abstract

Fermented vegetables and fruits are a good source of selected vitamins, antioxidants, microelements and revealed immune-boosting properties. The appropriate content of health-promoting compounds and probiotics allows you to qualify juices from fermented vegetables or fruits as better-for-you food. Notwithstanding, biogenic amines in this type of food are also detected due to the fermentation process, which may negatively affect their health-promoting properties. In this study, various biogenic amines were determined in juices obtained during the pressing of fermented vegetables (white and red cabbage, cucumbers, tomatoes, cauliflowers, celery, celery leaves, broccoli, and beetroots). The level of monamines (histamine, tyramine, tryptamine, and phenylethylamine), diamines (putrescine, cadaverine), and polyamines (spermidine and spermine) was discussed. The significant discrepancies between all the tested determined compounds as well as the sums of the BAs in tested samples, were observed. The calculated values of BAs sum varied from 6.36 $mg \cdot L^{-1}$ (fermented tomato juice) to 116.52 $mg \cdot L^{-1}$ (fermented cucumber juice). Among the determined amines, the majority were histamine, tyramine, putrescine, and cadaverine (from 66% to 97-98% of total biogenic amines level). Moreover, depending on the type of tested juice, the amount of histamine and tyramine

in the full content of BAs was between 0.44% (for fermented red cabbage juice) and 68% (for fermented broccoli juice). According to our knowledge, this is the first time that the determination of biogenic amines in fermented vegetable juices has been studied.

1. Introduction

The fermentation processes are widely applied in food industry for the production of food with new, unique flavours, textures, functionalities, and health values. The fermented products, mainly obtained using lactic acid bacteria (LAB), being simultaneously sources of probiotic-like microbes, resulting in health-promoting properties of food. LAB fermentation processes increase the number of vitamins, biologically active peptides, and antioxidant activity in vegetables and juices. The latter causes fermented food to become a more attractive component of a healthy diet. It has been reported that in Europe, at least 21 different vegetables are fermented, and an unspecified number of vegetable blends and fermented vegetable juices are available on the food market (Ashaolu & Reale, 2020). For this reason, dietary and natural health promotion organisations recommend fermented food for all over Europe's national diet components (Marco, et al., 2017). Moreover, fermented food reveals a longer shelf-life than raw one that is vital for food waste reduction on the continental scale.

Due to promoting human health, fermented vegetables, and fruits can be considered functional foods targeted at younger and older consumers (Plessas, 2022). It should be added that probiotics-based foods are the fastest-growing field in functional food production (Lillo-Perez, et al., 2021). Besides, it is recognized that the consumption of probiotics has beneficial effects on the prevention of various diseases. Single microbial strains or multi-strain probiotics can be immune-boosting against the COVID-19 virus (Singh & Rao, 2021).

Besides the positive impact of fermented food, there are some concerns regarding the possible negative impact of the significant levels of biogenic amines (BAs) associated with the activity of fermentative microorganisms. These microbial metabolites are formed by a decarboxylation of their precursor amino acids. They can be found in almost all types of food in a wide range of concentrations, which may vary even within the same product (Hernández-Macias, et al., 2022). In fermented food, monoamines, such as histamine (Him), tyramine (Tyr), phenylethylamine (Phen), tryptamine (Trp), and di- and polyamines, *e.g.*, putrescine (Put), cadaverine (Cad), spermidine (Spd) or spermine (Spm), were detected. Some of them (histamine and tyramine) are involved in human essential and various physiological processes. In contrast, Cad and Put are instead very toxic and responsible for the toxicosis caused by bacterial intestinal alterations (Mannino, et al., 2022). The ingestion of foods containing high

levels of BAs caused by excessive microbial decarboxylation determines several adverse effects on our health. It should be added that in recent years, good manufacturing practices and the use of non-aminogenic starter cultures or the use of LAB starter culture to control the accumulation of BAs in fermented food have contributed to minimize the levels of these compounds in fermented products (Wang, et al., 2021). However, in the case of fermented foods containing live cultures, the level of BAs is a challenge for the food industry. The BAs levels in traditional fermented foods were reviewed by Gao, et al. (2023) and Sivamaruthi, Kesika, & Chaiyasut (2021). The authors discussed the impact of exceeding BAs amounts on human health.

The accurate and rapid BAs detection methods are used to control these compounds' formation in fermented food. However, BAs determination in food is usually complicated due to high polarity and low concentrations in complex food matrices, which imposes some pre-processing steps for sample analysis (Zhu, et al., 2021). Several procedures of BAs determination in food have been reviewed recently (Ahangari, et al., 2021; Jaguey-Hernandez, et al., 2021; Jain & Verma, 2018; Moniente, et al., 2022; Tırıs, et al., 2023; Vasconcelos, et al., 2021). The literature study indicates a high-performance liquid chromatography (HPLC) with pre-column or post-column derivatization as the most often used technique.

Currently, fermented vegetable and fruit juices are new and promising approaches for functional and convenient products. Since the intake of foods with high levels of BAs can induce toxic or adverse effects, determining these compounds is essential for the food industry and consumers. In the literature, the problem of BAs analysis in fermented vegetables has become more often studied (Gao, et al., 2023; Hernández-Macias, et al., 2022; Świder, et al., 2020); however, there are less data available on BAs level in fermented vegetable juices.

For this reason, the main research goal was to determine important BAs, such as histamine, tyramine, tryptamine, phenylethylamine, putrescine, cadaverine, spermine, and spermidine in fermented vegetable juices commercially available on the pro-health food market. The chromatographic determination after a minimal sample preparation procedure and pre-column derivatization of BAs with *o*-phthalaldehyde (OPA) was applied.

The juices embossed from fermented, organic vegetables were the research material. The samples were obtained from one health food store and were produced by one manufacturer. The juices from the popular fermented vegetables: white and red cabbage, beetroot, and cucumber, and less popular fermented vegetables, such as celery and celery root, broccoli, cauliflower, and tomato, were analyzed. All tested samples can be treated as natural probiotics, a good source of vitamins, minerals, and antioxidant compounds. However, the level of BAs, due to the fermentation process, can also be high. Because there is a lack of regulatory levels

on the content of the BAs in fermented food and medical suggestions, particularly polyamines, in an organism, the study of composition is important. It provides new knowledge in the area of the healthy food market. To the best our knowledge determination of biogenic amines in fermented vegetable juices has not been studied yet.

2. Experimental

2.1. Sample

Biogenic amines were determined in fermented vegetable juices commercially available on the Polish pro-health food market. The tested products came from one producer and included: fermented celery root juice (sample 1), fermented celery juice (sample 2), fermented broccoli juice (sample 3), fermented cauliflower juice (sample 4), fermented red cabbage juice (sample 5), fermented celery with beetroot juice (sample 6), fermented beetroot juice (sample 7), fermented tomato juice (sample 8), fermented cucumber juice (sample 9), sauerkraut juice (sample 10), multi-vegetable fermented juice (sample 11).

The juices were obtained from organic vegetables by pressing, pasteurization, and poured into green glass bottles (300 mL).

2.2. Reagents and instruments

Analytical grade: cadaverine (Cad), putrescine (Put), spermine (Spm), spermidine (Spd), tyramine (Tyr), histamine (Him), tryptamine (Trp), 2-phenylethylamine, 99% (Phen), *o*-phthalaldehyde (OPA) were purchased from Sigma Aldrich (Poland). Tetrahydrofuran, THF (for HPLC), methanol, MeOH (for HPLC), sodium acetate, sodium hydroxide, and 2-mercaptoethanol (MCE) were purchased from Alchem (Poland).

The HPLC system (Shimadzu Corp, Kyoto, Japan) was equipped with an autosampler SIL-20AC HT and a fluorescent detector (RF-20Axs; Shimadzu Corp, Kyoto, Japan) applied. Analyses were carried out on a Gemini 5 μm NX-C18 LC Column 250/4.6 (Phenomenex LTD Deutschland). The LC solution program version 1.23 SP recorded and processed the chromatographic data. The laboratory centrifuge (MPW-350, Poland, max speed 9000 rpm, RFC 8693xg, angle 30°, falcon tubes 50 mL) was used for sample centrifugation. The pH measurements were made with a multifunctional computer meter (Elmetron, Poland).

2.3. Preparation of derivatization reagent

The OPA solution (0.05 M) was prepared with 335 mg *o*-phthalaldehyde (2.5 mmol) dissolved in 5 mL MeOH, and then 270 μL 2-mercaptoethanol (3.75 mmol) was added. OPA

reagent solution was diluted to a volume of 50 mL in a volumetric flask with carbonate buffer (pH=10). This solution can be stored in the refrigerator (4 °C) for a week.

2.4. OPA-BAs derivatives synthesis

All reactions were carried out for 0.05 mmol BAs. For BAs with one primary amino group: 2-phenylethylamine (Phen), tyramine (Tyr), tryptamine (Trp), and histamine (Him), 2 ml of OPA reagent solution was added. In the case of BAs with two primary amino groups: cadaverine (Cad), putrescine (Put), spermine (Spm), and spermidine (Spd), 4 mL of OPA reagent solution to BAs was added. The mixture was stirred for 15 min at room temperature. After the reaction, the mixture was diluted with MeOH in a volumetric flask (25 mL) and was diluted 10 times.

2.4. Sample preparation

In the case of fermented vegetable juices, derivatization reactions were carried out in the same way: 5 mL of the samples were mixed with sodium hydroxide solution (1 M) until the pH = 10, and 1 mL OPA reagent solution was added. The mixture was stirred for 15 min at room temperature, centrifuged (9000 rpm; 15 min), filtrated, and diluted with MeOH in a volumetric flask (10 mL).

2.5. Chromatographic analysis

Obtained derivatives of BAs were determined using RP-HPLC. The mobile phase was: 5 mM sodium acetate/tetrahydrofuran 96:4, v/v (solvent A) and methanol (solvent B), and the gradient conditions were: 0-4 min A: 47%; 4-9 min A: 30%; 9-16 min A: 0%; 16-30 min A: 42%, and 30-45 min A: 47. The total flow rate – 1.2 mL/min; the injection volume – 20 μL. The obtained derivatives were detected using a fluorescence detector (excitation wavelength of 335 nm and emission wavelength of 450 nm).

2.6. Statistical evaluation of procedure

The developed procedure was evaluated to determine the linearity, coefficient of determination, detection and quantification limits (DL and QL), precision (coefficient of variation, CV), accuracy (recovery studies), and matrix effect (ME). Procedure accuracy was calculated by standard addition method applying five replicates analyses of BAs mixtures at different concentration levels. The matrix effect was calculated by additions of BAs standard solution in samples of five different juices (Jia, et al., 2011). The standard addition slopes were

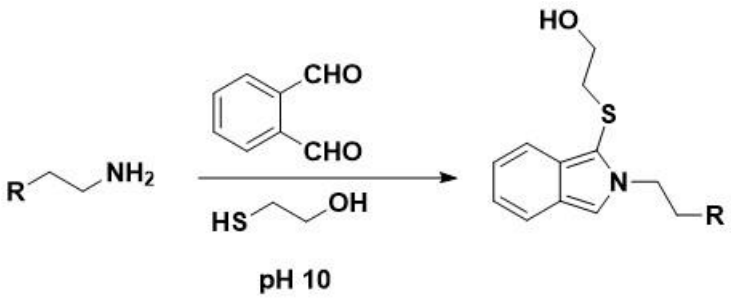
calculated by least-square linear regression for standards and food samples, whereas ME by computing the ratio of tested food samples slopes to standard solution slope.

For each tested juice, five samples were used for derivatization procedures and analyzed by HPLC in triplicate. One-way ANOVA followed by Duncan's multiple comparison tests was performed to determine the significant differences between data. A p-value less than 0.05 was assumed to indicate a substantial difference. All statistical data were obtained in the Statistica software, ver. 13 package.

3. Results and Discussion

o-Phthalaldehyde (OPA) reacts selectively and rapidly with primary amines in the presence of a thiol forming strongly fluorescent derivatives with concomitant satisfactory sensitivity of the method. Among disadvantages of this reagent, no reaction with secondary amines, low stability of obtained products were emphasized. Moreover, the obtained derivatives require strict conditions for formation, and do not allow for clean up after the derivatization (Jain & Verma, 2018). Nevertheless, OPA has been widely used for pre-, on-, or post-column derivatization of BAs and has been preferably detected by FLD (Moniente, et al., 2022). Its applicability is related to the creation of derivatives detectable by UV absorption spectrophotometry (at ~215 nm) and emission fluorescence spectroscopy (λex/λem, ~340–350/~420–450 nm), which results in very different conditions of synthesis (Hernández-Cassou & Saurina, 2011).

In this study, the following reaction conditions were applied: pH ~10 and stirring for 15 minutes at room temperature with the addition of mercaptoethanol (MCE), which resulted in stable cyclic derivatives. In the case of polyamines, a two-fold excess of the OPA should be used (**Fig 1**). The systematic names of obtained compounds are presented in **Fig 2**.



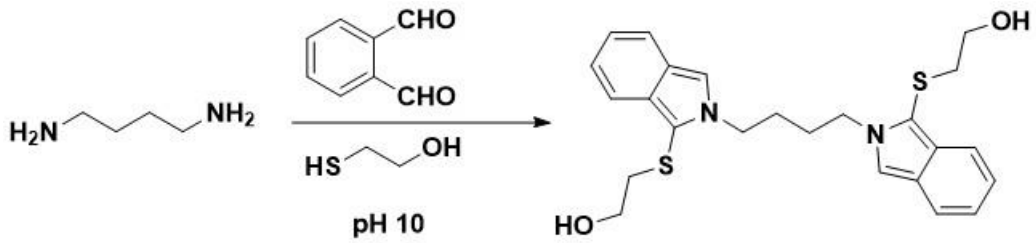


Fig 1. Reaction scheme of OPA with biogenic amines

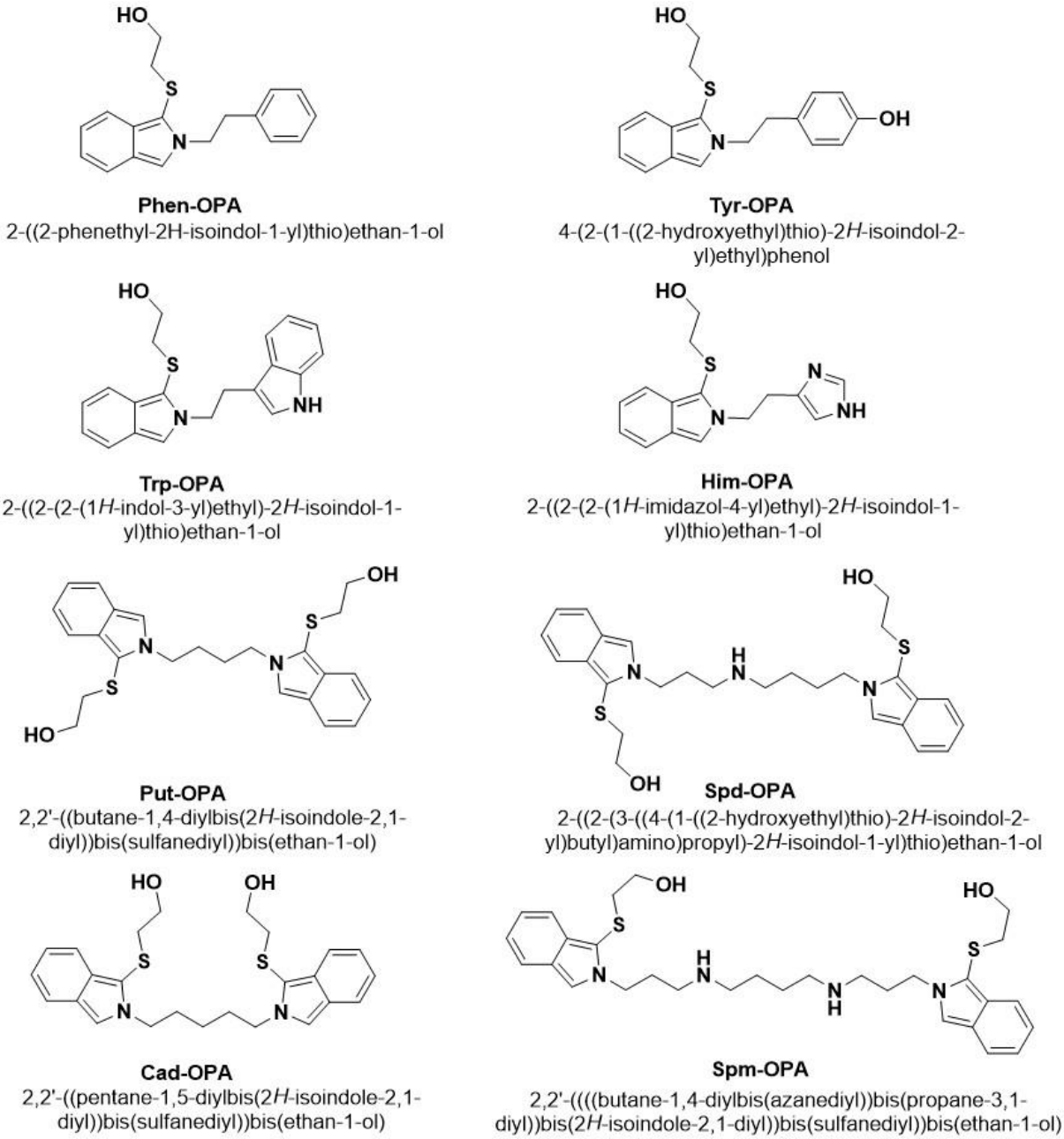


Fig. 2. The structural formulas of obtained BAs derivatives with OPA

3.1. The statistical evaluation of HPLC procedure

The selected BAs (Him, Tyr, Trp, Phen, Cad, Put, Spm, and Spmd) were determined in twelve fermented vegetable juices by RP-HPLC-FLD method based on pre-column OPA derivatization and a polarity gradient. The chromatogram of BAs derivatives standard solutions is presented in Fig. 3.

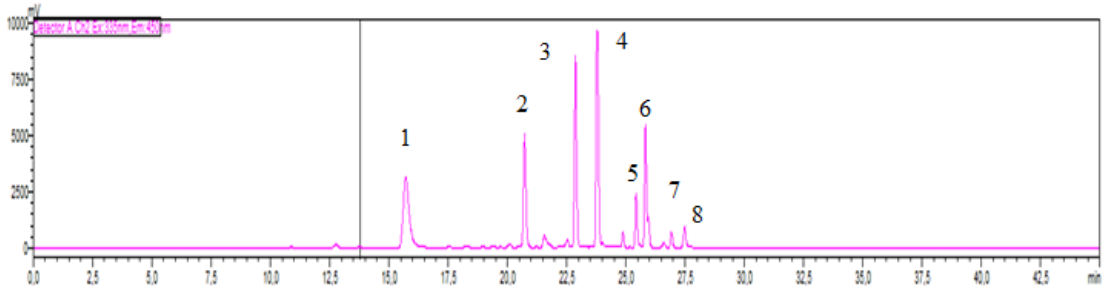


Fig. 3. HPLC chromatogram of the OPA derivatives of biogenic amines standard solution, where: 1 – Him-OPA, 2 – Tyr-OPA, 3 – Trp-OPA, 4 – Phen-OPA, 5 – Put-OPA, 6 – Cad-OPA, 7 – Spd-OPA, 8 – Spm-OPA

The statistical evaluation of the developed procedure: regression parameters of calibration curves for BAs, accuracy, precision, and matrix effect are listed in Table 1.

Table 1

The results regarding the linearity of the procedure, including its coefficient of determination, indicate a satisfactory linearity within the concentration range of studied BAs. Moreover, DL and QL revealed statistically acceptable values. The accuracy of the procedure was tested by the recovery studies of the BAs standard solution, and the average recovery index varied between 95.52-96.74% (more data in Supplementary Materials - Table S1). Moreover, the tested standard addition method for food samples exhibits recovery ranging from 94.16% to 97.39%, indicating satisfactory accuracy (more data in Supplementary Materials - Table S2). In order to evaluate the matrix effect, the slope comparison method was examined (Jia, et al., 2011). The slope ratio of the matrix curve to the standard solutions curve was calculated from 0.78 (Phen) to 0.97 (Him). Because the obtained values were close to 1.0, one can assume that the lack of significant matrix effects was observed using an elaborated procedure.

The CV values for the standards addition method were in the 0.99-2.70% range, indicating satisfactory intra-day reproducibility.

3.2. Determination of BAs in fermented vegetables juices

The results for samples of fermented vegetable juices are listed in Table 2. Moreover, a heat map depicting the average values grouped by samples and after correlation is presented in Fig. S1 (Supplementary Materials).

Table 2

For tested samples, we observed significant discrepancies between all the tested determined compounds and the sums of the BAs. The calculated values of BAs sum varied from 6.36 $mg \cdot L^{-1}$ (sample 8, fermented tomato juice) to 116.52 $mg \cdot L^{-1}$ (sample 9, fermented cucumber juice). The type of treatment of a raw material, availability and variety of amino acids, the presence of positive decarboxylase microorganisms, and storage time and temperature can justify these variations. According to our knowledge, no literature on BAs contents in fermented vegetable juices is available, whereas only limited literature on BAs in fermented vegetables is accessible (Majcherczyk & Surówka, 2019, Świder, et al., 2020). Herein, fermented cucumber juice and multi-vegetable fermented juice were characterized by the highest BAs concentration, while fermented tomato and fermented celery juices showed the lowest ones. However, such a low average content of BAs in fermented tomato juice is surprising. For comparison, Świder, et al. (2020) tested BAs contents in different varieties of fermented vegetables available in the Polish retail market. The total level of BAs in tomatoes varied from 15.30 $mg \cdot kg^{-1}$ to 117.14 $mg \cdot kg^{-1}$. As stated by the producer information, all tested by us juices were obtained in the process of pressing fermented vegetables and then pasteurized. Nevertheless, their composition may vary slightly depending on the time of year. Surprisingly, the content of eight tested BAs in sauerkraut juice (sample 10) was at a lower level than that observed for fermented cauliflower juice (sample 4) and fermented red cabbage juice (sample 5). The data obtained by Świder, et al. (2020) for total BAs in cauliflower and red cabbage were 50.48-702.09 $mg \cdot kg^{-1}$ and 49.18-700.45 $mg \cdot kg^{-1}$, respectively.

According to Jaguey-Hernández, et al. (2021), BAs can cause health problems due to high concentration in food or lower detoxification capacity due to gastrointestinal diseases, genetic predisposition, consumption of acetaldehyde derivatives present, *e.g.*, in wine, or medical treatments with monoamine oxidase (MAO) or diamine oxidase (DAO) inhibitors. Despite the knowledge of the negative impact of BAs on health, the limited standards of BAs in food are not unified in different countries and regions of the EU. Moreover, the toxicity thresholds of BAs in fermented foods have not been clearly defined. It is associated with the fact that the toxic dose of BAs is related to detoxification mechanisms, different individual

tolerance, and lifestyle habits (Gao, et al., 2023). Considering the above, the determined content of BAs, especially in fermented cucumber and multi-vegetable juices, could potentially be unfavourable for our health. On the other hand, US Food and Drug Administration (FDA) recommends a limit for the sum of BAs in food below 1000 $mg \cdot kg^{-1}$ (Gao, et al., 2023). Considering the latter, the total contents of tested biogenic amines in examined fermented vegetable juices are well below the FDA recommendations. However, the assessment of BAs impact on human health should consider daily intake, synergy effect, and some personal features.

Histamine and tyramine are considered the most critical BAs from a toxicological point of view by the European Food Safety Authority (EFSA) (Jaguey-Hernández, et al., 2021). The levels of 25 to 50 mg of histamine and 600 mg of tyramine per meal have no adverse effect on a healthy person (Majcherczyk & Surówka, 2019). This study found histamine and tyramine in almost all samples except sample 8 (fermented tomato juice) and sample 11 (multi-vegetable fermented juice), respectively. The noticeably high level of histamine was determined in the multi-vegetable fermented and fermented celery with beetroot juices. In contrast, a higher level of tyramine was in the sauerkraut and fermented celery juices.

Moreover, noticeable differences between tested samples were observed. For example, multi-vegetable pickled juice exhibits the highest histamine level, whereas tyramine concentration was below the detection limit. BAs concentration in sauerkraut was studied most frequently, possibly due to its popularity in Europe, especially in the Central and Eastern regions (Cvetković, et al., 2015; Majcherczyk & Surówka, 2019). Świder, et al. (2020) in sauerkraut determined average values of Him and Tyr on the level: 55.60 $mg \cdot kg^{-1}$ and 60.69 $mg \cdot kg^{-1}$, respectively.

Considering EFSA proposed levels of histamine and tyramine per meal (Majcherczyk & Surówka, 2019), the determined content of these amines in fermented vegetable juices is below the limit. However, depending on the type of tested juice, the amount of histamine and tyramine in the total content of BAs was between 0.44% (for fermented red cabbage juice) and 68% (for fermented broccoli juice). It should be pointed out that Him is also a mediator of allergic disorders. According to Ruiz-Capillas and Herrero (2019), BAs are released by mast cell degranulation (in response to an allergic reaction) and consuming foods containing histamine can have the same effect. Moreover, histamine detoxification systems can be hindered by various factors or circumstances, such as consuming amine oxidase inhibitors, immune deficiency of the consumer, or large amounts of BAs presented in fermented food. At

the time, histamine and other BAs could accumulate in the body, causing toxicological problems.

Today, one of the reasons for BAs analysis in food is observed an increased number of people taking antidepressants, such as monoamine oxidase inhibitors or diamine oxidase inhibitors drugs causing even minimal BAs content to be dangerous to patients (Givanoudi, et al., 2023).

Discussing content of remaining monoamines, we can note that Phen was found only in four samples (below 1 $mg \cdot L^{-1}$), whereas, for Trp, concentration ranges were wider. Fermented cucumber had a significantly higher tryptamine content than other samples of the tested juices.

It has been reported that histamine and tyramine toxicity is enhanced by putrescine and cadaverine, which decrease the catabolism of Him, favouring intestinal absorption and hindering histamine detoxification (Ruiz-Capillas & Herrero, 2019). Moreover, Put and Cad react with nitrite ions (food additive) and produce nitrosamines (1-nitrosopyrrolidine from Put and 1-nitrosopiperidine from Cad) at higher temperatures. However, for these amines suggested limit was not proposed due to the lack of sufficient data (Jaguey-Hernandez, et al., 2021). Herein, putrescine was found in all fermented vegetable juices, whereas Cad was noted in selected samples. The latter is beneficial because cadaverine is a potent DAO inhibitor and a weak histamine-N-methyltransferase inhibitor (Givanoudi, et al., 2022). Putrescine occurs naturally in plants and is most frequently present in fermented food. Significant levels of putrescine were observed in fermented cauliflower, red cabbage, cucumber, and multi-vegetable juices. For comparison, the minimal and highest values obtained by Świder, et al. (2020) for fermented cauliflower, red cabbage, and cucumber were as follows: 103.13-286.88 $mg \cdot kg^{-1}$, 4.00-278.95 $mg \cdot kg^{-1}$, and 26.75-158.35 $mg \cdot kg^{-1}$, respectively. The calculated sum levels of Him, Tyr, Put, and Cad are listed in Table 3.

Table 3

It should be added that the calculated sum of all BAs and the sum of Him + Tyr + Put + Cad is positively correlated (Pearson correlation coefficient = 0.9601), suggesting that these amines have the most substantial share in the total content of the analysed BAs. Only for fermented cucumber juice, Trp (36.29 $mg \cdot kg^{-1}$) is an amine that significantly increases the entire amount of BAs, which can have toxic effects on humans (blood pressure rises, hypertension) (Ruiz-Capillas & Herrero, 2019).

The share of Him + Tyr + Put + Cad in the sum of BAs contents in samples of beverages varied from 66% (fermented cucumber juice) to 97-98% (multi-vegetable fermented juice and fermented broccoli juice). The obtained results are similar to those obtained by Świder, et al. (2020).

Nevertheless, according to Tsafack and Tsopmo (2022), aromatic amines at a safe concentration level and hydrocinnamic acid derivates of aromatic and aliphatic BAs may enhance food stability by acting as antioxidant or antimicrobial agents. Moreover, Put, Spm, and Spd are essential in cell growth and differentiation. These compounds are naturally present at low concentrations in living organisms and foods, and sometimes additional polyamine doses are necessary for proper metabolic synthesis (Teratani, et al., 2021). It was reported that putrescine, spermidine, and spermine, as easily protonated, affect functions of nucleic acids (DNA, RNA) via strong ionic interactions resulting in DNA and RNA damage suppression, which may contribute to anti-aging effects (Givanoudi, et al., 2023). Exogenously, ingestion of polyamines protected against age-related memory loss and rescued memory performance (Khan, Chen & Geiger, 2021). Moreover, polyamines are critical to coronavirus replication and represent a promising drug target in current and future coronavirus outbreaks (Firpo, et al., 2021). Spermidine and spermine both inhibited SARS-CoV-2 infection and appeared to do so by inducing viral degradation in endolysosomes (Tsafack & Tsompo, 2022). Notwithstanding, the accumulation of spermine and spermidine in the organism can reverse their benefits and even lead to nephrotoxic and spermicidal effects (Givanoudi, et al., 2023).

Since polyamines have remarkable antioxidant activity, contributing to the elimination of free radicals generated during the metabolic processes, some of the tested juices can be considered a good source of these compounds (Table 3). The calculated Pearson correlation coefficient between the sum of BAs and the sum of polyamines was 0.8232, whereas between BAs sum and Put, 0.8248. The latter suggests that Put is the primary amine affecting the total content of the compounds tested.

For this reason, special attention should be paid to the level of spermidine and spermine in food. Spd is not discussed in the context of the negative impact on our health. Herein, the level of Spd in our samples varied from 0.37 $mg \cdot L^{-1}$ (multi-vegetable fermented juice) to 2.99 $mg \cdot L^{-1}$ (fermented cucumber juice). Spermidine is present in all plant-derived foods and is the predominant polyamine in green vegetables. Spm was observed only in selected juices from fermented cauliflower, celery with beetroot, beetroot, cucumber, and multi-vegetables at a lower level than other polyamines.

The correlation heat map (Supplementary Materials, Fig S1) of all food samples indicates the positive calculated correlation (calc. corr.) between polyamines: Cad - Spm, Cad - Spm (calc. corr. ~0.7), Put - Cad (calc. corr. ~0.3), Put and Spm (calc. corr. ~0.1). The calculated negative correlation between Him and Spd ~(-)0.5 suggest that spermidine levels increased along with the lower histamine content. A similar correlation of ~ (-)0.3 was observed between spermidine and spermine. The above discussion confirms that further research on BAs content in pickled vegetable juices is worth studying.

4. Conclusion

Biogenic amines are essential for human health; however, high food levels are associated with toxic effects. Histamine and tyramine are considered unhealthy for our health; however, it is difficult to propose toxicity levels for BAs contents due to numerous factors that can affect their toxicity. In the tested eleven samples of fermented vegetable juices, statistically significant differences between particular determined BAs and sums of them were observed. Calculated values of BAs sum varied from 6.36 $mg \cdot L^{-1}$ (fermented tomato juice) to 116.52 $mg \cdot L^{-1}$ (fermented cucumber juice). The amount of histamine and tyramine in the total content of BAs ranged from 0.44% (fermented red cabbage juice) to 68% (fermented broccoli juice), depending on the raw material. Histamine, tyramine, putrescine, and cadaverine exhibit the leading share in the total amount of determined amines. The sum of these amines varied from 66% to 97-98% of the entire content of biogenic amines. Obtained results of BAs determination indicate that fermented juice should not be undesirable for an average consumer.

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CRediT authorship contribution statement

Aneta Jastrzębska: Conceptualization, Data curation, Investigation, Methodology, Visualization, Supervision, Writing—original draft, Writing—review and editing. **Anna Kmieciak**: Conceptualization, Investigation, Methodology, Visualization, Writing—original draft, Writing—review and editing. **Kamil Brzuzy:** Investigation, Writing—original draft. **Zuzanna Gralak:** Investigation, Writing—original draft. **Marek P. Krzemiński:** Conceptualization**,** Investigation, Writing—original draft, Writing—review and editing. **Edward Szłyk:** Supervision, Writing—original draft, Writing—review and editing.

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Data availability

The discussed data are based on test results from Authors under the grant of National Science Centre, Poland [grant number 2020/39/B/NZ9/01694]. The data can be shared.

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Table 1. Linear regression calibration parameters of BAs determination, accuracy, precision and matrix effect

BAs	t_R X ± SD [min]	Linear range [mg·L^{-1}]	R^2	DL [mg·L^{-1}]	QL [mg·L^{-1}]	Mean recovery[1] [%]	Mean recovery[2] [%]	CV[2] [%]	ME
Him	15.69 ± 0.30	0.02-8.31	0.9994	$0.05 \cdot 10^{-1}$	0.02	96.32	97.03	1.29	0.97
Tyr	20.43 ± 0.07	0.02-11.63	0.9988	0.01	0.04	95.52	95.65	0.97	0.95
Trp	22.69 ± 0.20	0.03-14.67	0.9994	0.01	0.04	96.38	97.39	1.64	0.96
Phen	23.69 ± 0.04	0.02-9.42	0.9994	$0.08 \cdot 10^{-1}$	0.02	96.54	94.16	1.63	0.78
Put	24.77 ± 0.78	0.01-3.31	0.9992	$0.06 \cdot 10^{-1}$	0.02	96.74	94.84	1.67	0.96
Cad	25.55 ± 0.05	0.01-4.32	0.9984	$0.04 \cdot 10^{-1}$	0.01	95.96	95.07	2.05	0.93
Spd	26.50 ± 0.08	0.02-8.01	0.9994	$0.06 \cdot 10^{-1}$	0.02	96.00	96.85	0.99	0.94
Spm	27.64 ± 0.02	0.03-8.41	0.9987	0.01	0.03	96.21	95.57	2.70	0.84

Where: DL - detection limit; QL - quantification limit; CV - coefficient of variation; ME - matrix effect; [1] - standard solution; [2] - food samples

DL = 3.3×(Sy/x·a-1) and QL = 10×(Sy/x·a-1), where Sy/x is the standard deviation of the incept of the calibration curve, and a is the slope of the calibration curve

ME = (mean slope for standard addition curves/slope in the standard solution calibration curve)

Table 2. Results of biogenic amines determination **(X±SD) [mg·L^{-1}]** in fermented vegetables juices samples after derivatization with OPA

	Him	**Tyr**	**Trp**	**Phen**	**Cad**	**Put**	**Spm**	**Spd**	**Sum of BAs**
Sample 1	**12.31^{F}** ±0.23	**8.29^{G}** ±0.16	**0.23^{A}** ±0.01	nd	**8.41^{B}** ±0.10	**6.07^{B}** ±0.03	nd	**2.69^{I}** ±0.03	**38.00**

Sample 2	**1.76^{B}** ±0.04	**2.58^{E}** ±0.03	below DL	nd	below DL	**4.89^{A}** ±0.04	nd	**1.77^{G}** ±0.15	**11.00**
Sample 3	**13.47^{G}** ±0.20	**4.63^{F}** ±0.09	below DL	below DL	**2.27^{A}** ±0.29	**5.82^{B}** ±0.07	nd	**0.46^{B}** ±0.01	**26.65**
Sample 4	**0.026^{A}** ±0.001	**1.16^{C}** ±0.07	**1.07^{B}** ±0.03	**0.083^{A}** ±0.004	below DL	**82.5^{I}** ±0.55	**0.011^{A}** ±0.003	**2.80^{J}** ±0.01	**87.65**
Sample 5	**0.186^{A}** ±0.003	**0.117^{A}** ±0.002	**1.05^{B}** ±0.01	**0.542^{B}** ±0.014	**2.14^{A}** ±0.09	**61.98^{H}** ±0.02	nd	**2.38^{H}** ±0.01	**68.40**
Sample 6	**22.03^{H}** ±0.46	**2.31^{D}** ±0.05	**2.09^{C}** ±0.01	**0.872^{C}** ±0.010	below DL	**12.85^{C}** ±0.01	**0.145^{D}** ±0.002	**0.97^{D}** ±0.01	**41.27**
Sample 7	**4.30^{C}** ±0.16	**0.53^{B}** ±0.02	**2.47^{C}** ±0.09	below DL	**9.89^{C}** ±0.08	**14.25^{D}** ±0.27	**0.050^{B}** ±0.001	**1.545^{F}** ±0.002	**33.04**
Sample 8	nd	**0.116^{A}** ±0.005	**0.690^{A,B}** ±0.01	nd	nd	**4.71^{A}** ±0.01	nd	**0.84^{C}** ±0.01	**6.36**
Sample 9	**8.41^{E}** ±0.38	**0.069^{A}** ±0.002	**36.29^{E}** ±1.65	**0.072^{A}** ±0.003	**13.02^{D}** ±0.33	**55.61^{G}** ±0.39	**0.061^{C}** ±0.004	**2.99^{K}** ±0.01	**116.52**
Sample 10	**7.70^{D}** ±0.22	**10.69^{H}** ±0.06	**8.26^{D}** ±0.07	nd	below DL	**37.37^{E}** ±0.03	nd	**1.04^{E}** ±0.01	**65.06**
Sample 11	**29.43^{I}** ±0.45	below DL	**1.89^{C}** ±0.08	nd	**29.92^{E}** ±0.38	**41.68^{F}** ±0.03	**0.234^{E}** ±0.005	**0.37^{A}** ±0.02	**103.52**

Where: sample 1 - fermented celery root juice, sample 2 - fermented celery juice, sample 3 - fermented broccoli juice, sample 4- fermented cauliflower juice, sample 5 - fermented red cabbage juice, sample 6 - fermented celery with beetroot juice, sample 7 - fermented beetroot juice, sample 8 - fermented tomato juice, sample 9 - fermented cucumber juice, sample 10 - sauerkraut juice, sample 11 - multi-vegetable fermented juice

X ± SD - mean value ± standard deviation;

Different letters (A–K) within the same column indicate significant differences (one-way ANOVA and Duncan test, $p<0.05$); sorted from the lowest to highest values, where "a" was the lowest

Table 3. The sum of Him + Tyr + Put + Cad and polyamines

	Sum of Him + Tyr + Put + Cad	Sum of Put + Spd + Spm
Sample 1	35.08	8.76
Sample 2	9.23	6.66
Sample 3	26.19	6.28
Sample 4	83.69	85.31
Sample 5	64.42	64.36
Sample 6	37.19	13.97
Sample 7	28.97	15.85
Sample 8	4.83	5.55
Sample 9	77.11	58.66
Sample 10	55.76	38.41
Sample 11	101.03	42.28

SUPPLEMENTARY MATERIALS

Table S1. Accuracy and precision of the standard solutions determination

	Concentration [mg·L^{-1}]	Found concentration [mg·L^{-1}]	CV [%]	Recovery [%]
	0.050	0.048	0.67	95.84
	0.33	0.32	0.22	97.68
Him	0.83	0.79	4.09	95.64
	1.66	1.57	1.53	94.35
	4.57	4.48	0.40	98.10
	0.070	0.063	6.14	90.89
	0.46	0.44	2.41	95.48
Tyr	1.74	1.69	3.59	97.23
	2.33	2.29	2.52	98.39
	6.98	6.67	0.68	95.62
	0.088	0.083	3.01	94.73
	0.59	0.56	4.99	95.14
Trypt	1.47	1.40	0.17	95.20
	2.93	2.91	0.91	99.29
	5.65	5.51	0.89	97.53
	0.056	0.054	2.38	96.19
	0.38	0.35	1.86	94.50
Phen	0.94	0.92	1.09	97.63
	2.82	2.73	0.39	96.83
	5.65	5.51	0.89	97.53
	0.020	0.019	3.36	97.08
	0.14	0.13	1.28	96.26
Put	0.33	0.31	2.85	94.81
	0.66	0.64	0.79	96.77
	3.31	3.27	0.30	98.77

	0.026	0.025	3.16	95.12
Cad	0.17	0.16	4.10	95.90
	0.43	0.41	2.80	96.26
	0.86	0.82	0.75	95.56
	4.32	4.19	3.38	96.95
	0.048	0.044	2.14	92.83
	0.32	0.30	3.63	95.29
Spmd	0.80	0.77	1.00	96.82
	1.60	1.58	1.22	98.73
	4.81	4.63	1.05	96.31
	0.084	0.079	3.78	93.78
	0.56	0.55	1.87	98.26
Spm	1.40	1.34	2.48	95.79
	2.10	2.01	0.61	95.88
	7.01	6.93	3.70	97.36

Where: CV - coefficient of variation

Each solution was analysed five times

Table S2. Accuracy and precision for standard addition method (n=3)

BAs	Added concentration [mg·L^{-1}]	Recovery [%]	CV [%]
Him	1.25	95.84	0.70
	2.91	96.79	0.64
	4.57	98.46	0.45
Tyr	1.74	95.85	1.74
	4.07	95.49	0.62
	6.40	95.61	0.49
Trypt	2.20	95.68	0.72
	5.13	97.74	0.56
	8.07	98.76	1.45
Phen	1.41	93.34	0.90
	3.30	94.31	2.12
	5.18	94.83	1.82
Put	0.50	92.86	1.67
	1.16	93.46	2.15
	1.82	98.21	1.20
Cad	0.65	94.69	2.48
	1.51	95.03	2.11
	2.38	95.50	2.40
Spmd	1.20	94.87	1.08
	2.80	96.45	1.35
	4.40	99.22	0.54
Spm	2.10	96.44	0.61
	4.91	95.07	3.10
	7.71	95.20	4.39

Each solution was analysed five times

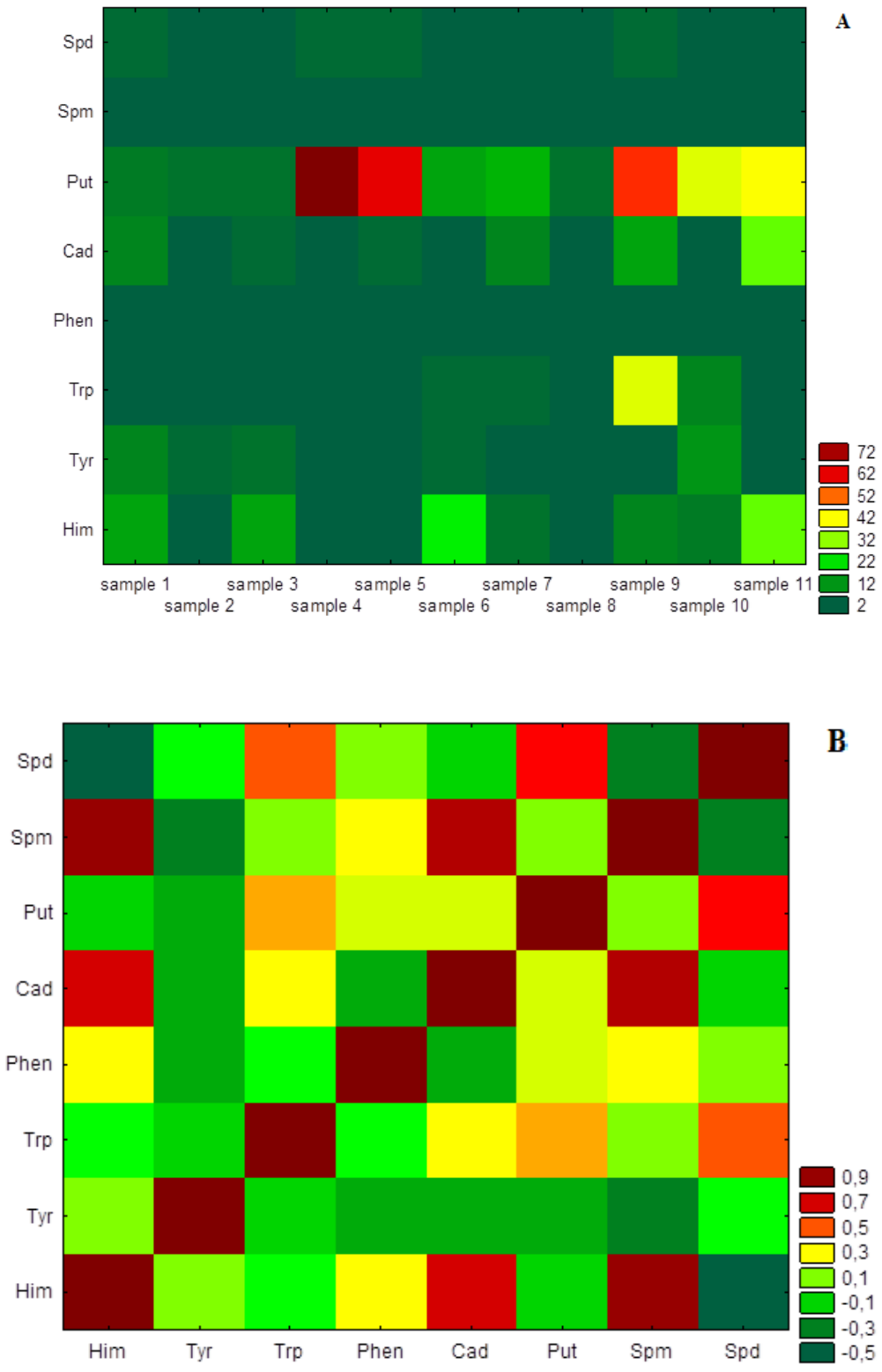

Fig S1. The heat map depicting the average values grouped by samples (A) and after correlation (B)