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Monitoring and risk assessment of biogenic amines in Korean commercial fermented seasonings

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ABSTRACT

Fermented seasonings are widely used in Korean cuisine; however, some contain high levels of biogenic amines (BAs). In this study, in order to estimate the potential BA risk from fermented seasoning, the BA contents in 50 commercial brands (n = 292) of five seasonings (soy sauce, *Gochujang, Doenjang, Ssamjang*, and *Cheonggukjang*) and their changes due to different cooking methods were evaluated by high-performance liquid chromatography. The risk assessment for consumer exposure was evaluated by computing the estimated daily intake (EDI), histamine (HIS) intake, and tyramine (TYR) intake. Maximum contents of HIS detected in *Cheonggukjang*, soy sauce, *Doenjang*, and *Ssamjang* were 318.46, 148.15, 123.65, and 114.07 mg/kg, respectively. However, even in the worst-case scenario, the EDI value and HIS intake results demonstrated that the seasonings had a limited impact on the risk of HIS poisoning due to their low consumption. *Cheonggukjang* exhibited the highest TYR content (312.89 mg/kg), and the TYR exposure results implied that those taking classical antidepressant monoamine oxidase inhibitors should pay attention to *Cheonggukjang*. After stir-frying, the total BA retentions in soy sauce and *Gochujang* were only 51.45% and 57.08%, respectively, which may be caused by high temperature. Based on the results of this study, the five seasonings contained various BAs, which can be influenced by the cooking process, and all five seasonings are safe for the general population in terms of the risk of BAs.

1. Introduction

Biogenic amines (BAs) are low-molecular-weight compounds required for a number of essential biological functions [1]. At low concentrations, BAs can act as hormones or neurotransmitters, but high concentrations of BAs lead to health problems, like hot flushes, heart palpitations, and headaches, and can have toxicological consequences [2]. Moreover, the pathogenesis of various neurological diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, or schizophrenia, has been linked to abnormal levels of biogenic amines in the central nervous system [3].

Major BAs found in foods are tryptamine (TRY), 2-phenylethylamine (PHE), putrescine (PUT), cadaverine (CAD), spermidine (SPD), spermine (SPM), histamine (HIS), and tyramine (TYR) [3]. The HIS and TYR levels are especially important when assessing food product risk because of their association with neurological and gastrointestinal diseases, like migraine, nausea, diarrhea, and blood pressure disorders [4]. The EFSA Panel on Biological Hazards (BIOHAZ) [5] set the acute reference dose (ARfD) of HIS at 25–50 mg/meal/person, and the no-observed-adverse-effect-level (NOAEL) for TYR was set at 600 mg/meal/person. It is also important to

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note that HIS is the main component responsible for scombroid poisoning. Other BAs, such as PUT and CAD, have been associated with this illness, although both appear to have substantially lower pharmacological activity on their own but enhance the adverse effects of HIS and TYR [6]. Moreover, PUT, CAD, SPD, and SPM can react with nitrite to facilitate the formation of probable or potential human carcinogens known as *N*-nitrosamines [7].

The formation of BAs in food mainly occurs as a result of microbial decarboxylation of the corresponding amino acids by substrate-specific decarboxylase enzymes [5]. Therefore, BAs are mainly found in fermented foods rich in protein [8]. Fermented seasonings are classical fermented foods and a popular product in the daily lives of Koreans. Variations in the microbial composition [9] and fermentation conditions [10], the types of salt [11], and the composition of the seasoning (e.g., combining fermented seasonings with foods rich in antioxidants) [12] can alter the BA levels in some Korean seasonings. Despite such findings, few studies have focused on the consumer exposure assessment of BAs in the above daily used seasonings in Korea. Instead, previous studies have tended to focus on the risk assessment of BAs in fish [13], sausage [14], and cheese [15]. *Doenjang* and *Cheonggukjang* are popular fermented soybean pastes in Korea. The mean contents of HIS and TYR in *Doenjang* and *Cheonggukjang* were reported as 170.11 and 260.99 mg/kg [16] and 8.37 and 457.42 mg/kg, respectively [17]. These values were higher than those reported in fish, sausage, and cheese [13–15]. Hence, a BA risk assessment for seasoning is necessary.

While the consumption of seasoning per meal is usually not as much as that of other food products, the frequency of use of seasonings is high in Korea, and seasonings have been an integral part of Korean food culture for centuries [18]. Therefore, it is important to calculate the risks associated with the consumption of BAs in seasonings per meal and per day.

According to the latest Korea National Health and Nutrition Examination Survey (KNHANES) database (2021; https://knhanes.kdca.go.kr/knhanes/main.do), an annual survey carried out by the South Korea Center for Disease Control and Prevention (KCDC), the most prevalent fermented seasoning in Korea is soy sauce, followed by *Gochujang*, *Doenjang*, *Ssamjang*, and *Cheonggukjang*. Hence, these most popular seasonings were selected for further assessment of the BA risk in this study.

In daily Korean life, seasonings are typically consumed after cooking, and, as mentioned above, the BA contents of food products can be affected by the cooking process [19]. Consideration of the cooking methods in the risk assessment can provide a more accurate and comprehensive evaluation of the potential risks associated with BAs in seasoning products. However, few reports have combined the influence of cooking with BA risk assessment. Therefore, in addition to analyzing the BA contents of soy sauce, *Gochujang*, *Doenjang*, *Ssamjang*, and *Cheonggukjang*, this study investigated the cooking effect in order to provide a comprehensive evaluation of their associated health risks.

In this study, 10 commercial brands for each seasoning (*Cheonggukjang*, *Doenjang*, soy sauce, *Gochujang*, and *Ssamjang*) were collected and cooked by typical cooking methods for Korean foods. The BA contents of samples were analyzed by high-performance liquid chromatography (HPLC), and based on the dietary database of Korean consumers, we also conducted a risk assessment of HIS and TYR in the tested seasonings to estimate the daily exposure.

2. Materials and methods

2.1. Chemicals

Dansyl chloride, TRY hydrochloride, PHE hydrochloride, PUT dihydrochloride, CAD dihydrochloride, HIS dihydrochloride, TYR hydrochloride, SPD trihydrochloride, and SPM tetrahydrochloride were all purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Perchloric acid, sodium hydrogen carbonate, L-proline, potassium dichromate, and silver nitrate were purchased from Daejung Chemical Co. (Siheung, Korea). Distilled water, acetone, and acetonitrile (HPLC grade) were purchased from Tedia Co. (Fairfield, OH, USA).

2.2. Sample collection

In this study, the most consumed fermented seasonings were selected based on the KNHANES database (2021; https://knhanes.kdca.go.kr/knhanes/main.do). We collected 10 samples each of *Doenjang* (D1−D10), soy sauce (S1–S10), *Gochujang* (G1−G10), *Cheonggukjang* (C1–C10), and *Ssamjang* (SS1−SS10) from local markets in the eight largest cities in Korea (Fig. S1) in 2021 by brand name, in accordance with their order of market share, with reference to the data in the Food Information Statistics System (https://www.atfis.or.kr/). One hundred grams of samples (same brand) collected from different cities were homogenized using a homogenizer (IKA, Staufen, Germany) to make a representative sample. After homogenization, samples were stored at −80 °C until analysis.

2.3. Sample preparation for cooking effect evaluation

The cooking methods were selected by matching each type of seasoning to a corresponding cooking method used in cooking ≥5% of the intake amount of each seasoning or with ≥5% frequency of use from the KNHANES database (2021). Two different cooking methods, boiling and stir-frying, were selected for soy sauce and *Gochujang*, and a single cooking method, boiling, was selected for *Cheonggukjang* and *Doenjang*. From the KNHANES database (2021), *Ssamjang* is usually eaten raw, so the effects of cooking were not tested on this seasoning.

Initially, with reference to the method reported by Kim et al. [20], 100 g of the 10 representative samples of each type of seasoning was homogenized to make composite samples, which also served as the control samples. Then, for boiling, according to the method of Shi et al. [21], 10 g of each sample was mixed with water in a conical flask to a final volume of 50 mL and boiled for 15 min. Stir-frying was performed following the method described by Kim et al. [20]. Samples were placed in a 170 °C preheated pan and stir-fried for 2 min. All samples were collected when cooled to room temperature (18–21 °C) and stored at −80 °C for further analysis.

2.4. BA analysis

2.4.1. Sample extraction and derivatization

The methods described by Shi et al. [21] for BA extraction from seasonings and chemical derivatization with dansyl chloride were used with slight modifications. Specifically, 5 g of each seasoning sample, except soy sauce, was vortexed with 20 mL of 0.4 M perchloric acid solution for 1 min and then placed in a refrigerator (4 °C) for 2 h. Afterward, the sample was centrifuged at 8000×*g* for 8 min at 4 °C, followed by mixing (in a vortex), extraction, and re-centrifugation of the residue. Finally, the two supernatants were pooled, and the volume was adjusted to 50 mL with 0.4 M perchloric acid solution. For soy sauce, 5 mL of the sample was directly diluted to 50 mL for further derivatization. All samples were filtered through an 11 μm filter paper (Advantech, Tokyo, Japan) for the derivatization of BAs.

2.4.2. HPLC analysis

The BA profile of seasonings was determined by HPLC analysis using a Waters Alliance 2695 Separation Module (Waters, Milford, MA, USA) with a photodiode array detector, a binary pump, and a vacuum degasser, based on the method published in our earlier paper [21]. Twenty microliters of sample solution was injected onto a C18 Supelco column (4.6 mm × 250 mm, i.d., 5 μm; Shiseido, Kyoto, Japan) thermostated at 35 °C. The mobile phase was 0.1 M ammonium acetate (solvent A) and acetonitrile (solvent B) with a flow rate of 0.5 mL/min, and the detection wavelength was 210 nm. The gradient elution procedure was as follows: 0 min, 35% A, 65% B; 5 min, 30% A, 70% B; 10 min, 19% A, 81% B; 15 min, 17.5% A, 82.5% B; 20 min, 100% B; 25 min, 35% A, 65% B; 35 min, 35% A, 65% B. Fig. 1 demonstrates the chromatograms of the standard and sample. The method validation was conducted as described previously [21], and the results are presented in Table S1.

2.5. Exposure assessment

In order to assess the risk of BA in the five types of fermented seasoning, an exposure assessment was calculated by two types of formulas [22].

2.5.1. Estimated daily intake (EDI) of BAs

The EDI values (mg/kg body weight [bw]/day) of seasonings in various age groups were calculated by Eq. (1):

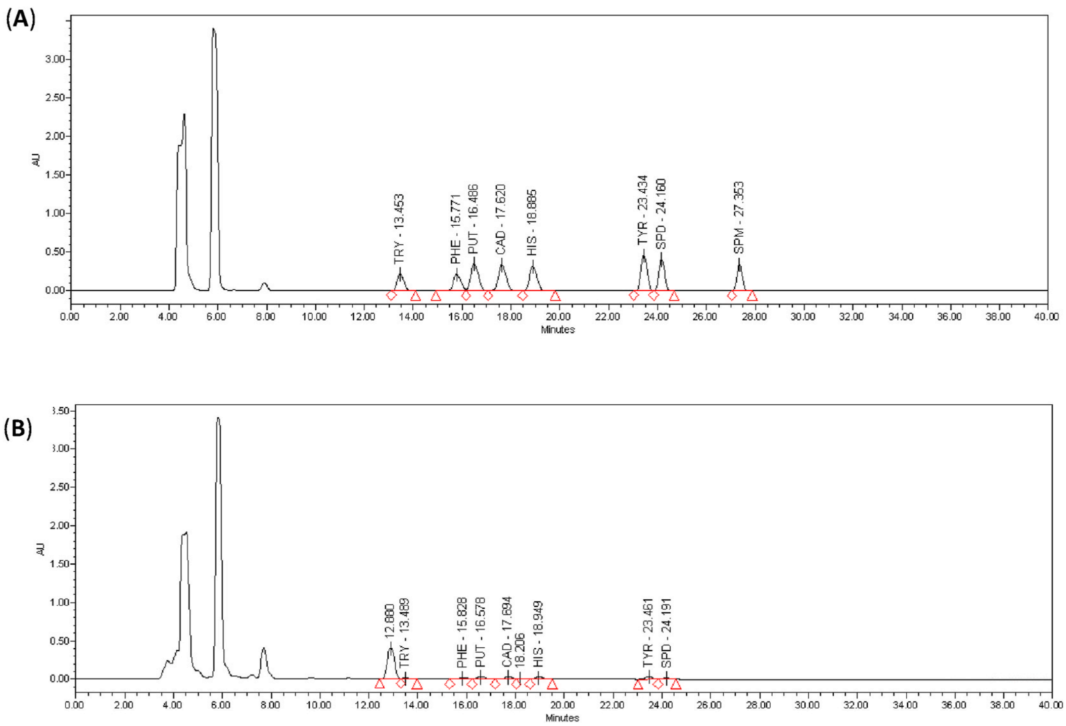

Fig. 1. HPLC chromatograms of standard (**A**) and sample (**B**).

$$\text{EDI}\left(\frac{\text{mg}}{\text{kg bw} \times \text{day}}\right) = C\left(\frac{\text{mg}}{\text{kg}}\right) \times M\left(\frac{\text{g}}{\text{kg bw} \times \text{day}}\right) \div 1000 \tag{1}$$

where C is the maximum BA concentration (worst-case scenario) in each seasoning, and M is the mean seasoning intake (quantity consumed per day). The consumption rate of seasonings for each age group in Korea was obtained from the latest KNHANES database (2021).

2.5.2. HIS and TYR risk assessment

In addition to the EDI values, the HIS and TYR intakes (mg/meal) were also calculated using the highest contamination concentration, as follows (Eq. (2)):

$$\text{Intake}\left(\frac{\text{mg}}{\text{meal}}\right) = C\left(\frac{\text{mg}}{\text{kg}}\right) \times F(\text{g} / \text{meal}) \div 1000 \tag{2}$$

where C is the maximum HIS or TYR concentration in each seasoning, and F is the intake per meal (one-third of the daily intake) of seasoning.

The value was compared with the ARfD (50 mg/meal) of HIS and the NOAEL of TYR.

2.6. Statistical analysis

All BA analysis data were displayed as the mean ± standard deviation from the computation of at least three measurements. Statistical analysis was performed using SPSS 24.0 (IBM Corp., Armonk, NY, USA). Significant differences were identified by performing a one-way analysis of variance (ANOVA), and Duncan's multiple range test was used to compare mean values at $p < 0.05$. The KNHANES database was analyzed using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) to obtain the daily intake for each seasoning. Principal component analysis (PCA) and heatmap diagrams were carried out using the online software MetaboAnalyst (https://www.metaboanalyst.ca/) version 5.0 (Xia Lab, McGill University, Quebec, Canada).

3. Results and discussion

3.1. BA concentrations in five types of seasoning

According to the validation results (Table S1), the HPLC method for BA determination demonstrated good precision, as all relative standard deviation values were lower than 2%. The recovery rates and matrix effect, which represent the accuracy, ranged from 83.05% to 118.43% (within the acceptable range of 80–120%) and −6.56% to 11.27% (within the acceptable range of −20% to 20%), respectively, indicating the reliability of the HPLC analysis method for BA in the five types of seasoning according to the SANTE 11312/2021 guidelines [23].

Table 1 summarizes the minimum, maximum, and median concentrations of BAs in five commercial fermented seasonings popular in Korean cuisine and chosen for analysis in this study. *Cheonggukjang* and soy sauce displayed the highest total BA contents (sum of the eight BA, ΣBA8), with ranges of 104.72–698.22 and 41.17−640.38 mg/kg, respectively, followed by *Doenjang* (83.38−269.90 mg/kg), *Ssamjang* (48.26−241.07 mg/kg), and *Gochujang* (15.43−86.06 mg/kg). The relatively lower use of soybeans during the production process of *Gochujang* might explain why it had the least concentration. The BA content of each commercial seasoning is shown in Table S2.

Table 1
Minimum, maximum, and median concentrations of biogenic amines (mg/kg wet weight) in 10 commercial samples of each fermented seasoning.

Seasonings	Measures	TRY	PHE	PUT	CAD	HIS	TYR	SPD	SPM	Total
Cheonggukjang	Minimum	9.35	*	*	15.00	3.35	18.15	*	*	104.72
	Median	42.55	*	4.61	19.79	38.14	116.16	*	9.26	285.41
	Maximum	59.22	60.27	25.13	32.29	123.65	312.89	47.44	182.67	698.22
Soy sauce	Minimum	*	*	1.75	8.21	11.25	*	*	*	41.17
	Median	11.50	70.98	15.34	18.74	58.33	20.05	*	10.44	231.76
	Maximum	73.34	91.09	69.89	48.86	318.46	114.41	7.34	54.59	640.38
Doenjang	Minimum	*	*	3.99	*	58.77	5.41	*	*	83.38
	Median	*	*	11.00	*	73.31	25.15	*	*	120.84
	Maximum	26.34	23.67	39.82	*	148.15	47.00	*	16.53	269.90
Gochujang	Minimum	*	4.85	3.10	*	*	*	*	*	15.43
	Median	4.35	5.89	7.21	5.35	*	1.23	*	*	23.72
	Maximum	11.56	11.81	19.20	7.86	8.38	16.24	9.51	14.83	86.06
Ssamjang	Minimum	*	*	*	*	*	*	*	*	48.26
	Median	*	*	11.85	18.13	33.70	*	*	*	104.15
	Maximum	76.93	28.33	46.23	24.59	114.07	13.45	15.47	23.38	241.07

TRY, tryptamine; PHE, 2-phenylethylamine; PUT, putrescine; CAD, cadaverine; HIS, histamine; TYR, tyramine; SPM, spermine; SPD, spermidine.
*Indicates the concentrations were below the respective limit of quantification (LOQ).

Considering the individual BAs, sample C4 had the highest TYR and SPM contents, and sample S4 exhibited the highest HIS concentration. For the other seasonings, the BAs in *Gochujang* were mostly detected at low concentrations (except for SPD), *Doenjang* had comparably high HIS and PUT contents, and *Ssamjang* mostly had high contents of TRY and CAD (Table S2). While these trends of BAs in *Doenjang* and *Gochujang* were the same as those observed by Kim et al. [16], some of the *Doenjang* samples showed different dominant individual BAs. There could be various reasons for this incongruence, such as the diverse ingredient sources, microbial population, fermentation environment, and salt content [24,25].

The PCA results for the BA contents in the five types of fermented seasoning are displayed in Fig. 2. Together, the first two principal components (PC1 and PC2) explained 80.1% of the total variance in the data set, contributing 55.0% and 25.1%, respectively. From the loadings plot, it was observed that all BAs were on the negative side of PC1. The main contributor to PC1 was TYR, followed by HIS. For PC2, TYR displayed the most extreme negative value, around −0.5, and HIS had the most extreme positive loading, over 0.8. The scores plot displayed the 95% confidence regions for the seasonings. Although no significantly separated group was observed in the figure ($p > 0.05$), it was noted that the majority of *Cheonggukjang* was on the negative side of both PC1 and PC2, indicating a higher TYR content in this seasoning than in the other seasonings, which was in agreement with a former report [9]. Soy sauce was mainly on the negative side of PC1 and the positive side of PC2, indicating that it had a higher HIS content than the other seasonings. Similarly, a previous study also reported that soy sauce has high levels of HIS and TYR, both with concentrations over 100 mg/L [26]. There was no distinguishable boundary between *Ssamjang* and *Doenjang*, as well as between *Ssamjang* and *Gochujang*, in the PCA plot. The similar BA

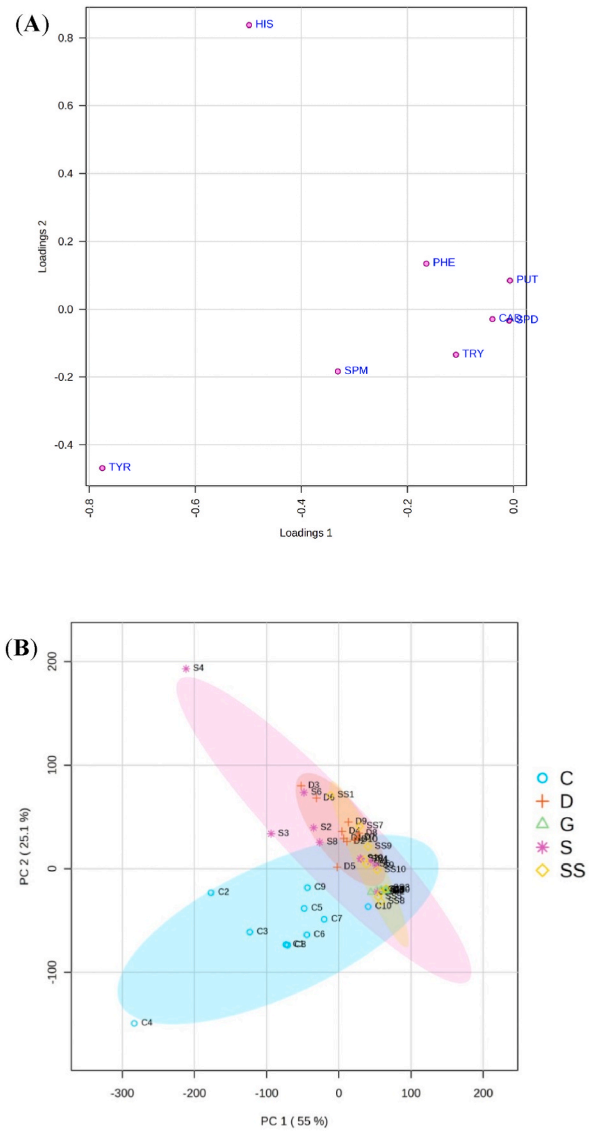

Fig. 2. PCA plots of biogenic amines in five fermented seasonings (**A**) loadings and (**B**) scores. C: *Cheonggukjang*; D: *Doenjang*; G: *Gochujang*; S: soy sauce; SS: *Ssamjang*.

contents of these seasonings may be because the main ingredients of *Ssamjang* were *Doenjang* and *Gochujang* [27].

3.2. Influence of cooking method on BA composition

The representative sample of each seasoning was cooked by the methods selected from the KNHANES database (2021); *Cheonggukjang* was boiled, soy sauce was boiled and stir-fried, *Doenjang* was boiled, and *Gochujang* was boiled and stir-fried. The changes in BA content before and after cooking were analyzed. As illustrated in Table 2 and Fig. S2, the percentage of the ΣBA8 retained after boiling was 95.99% for *Cheonggukjang* and 99.30% for *Doenjang*, respectively. This was consistent with the study by Yoon et al. [28], in which around 94% of the BAs in *Doenjang* were retained after boiling. The corresponding values for soy sauce and *Gochujang* were 85.72% and 89.48% (Fig. S2), respectively, after boiling and less than 60% each after stir-frying. It could be hypothesized that the least retention of BA in soy sauce after cooking is mainly because it is liquid and easier to evaporate than other seasonings [19]. For *Gochujang*, which contains more reducing sugars than the other seasonings [10,29–31], the greater decrease in the content of BAs after boiling might partly be due to the reaction of the amino groups of BAs with reducing sugars by the Maillard reaction [32]. Similarly, differences in the BA retention of a range of different foods after various culinary processes were also observed by Muñoz-Esparza et al. [33]. Additionally, it was found that stir-frying had a greater influence on the BA amounts compared to boiling. This could be attributed to the higher temperature reached during stir-frying, which can reach 170 °C, whereas boiling typically reaches approximately 100 °C. Given that the boiling points of BAs are all above 100 °C, it suggests that cooking by boiling is unlikely to cause the evaporation of BAs [19]. It should also be noted that, unlike boiling, which requires water as a medium, the direct contact between the sample and the hot plate when stir-frying would degrade the BAs within seconds. Similar results have been published in earlier reports. Polak et al. [19] showed that the total BA content (PUT, CAD, TYR, SPD, SPM) in bread crust was lowered at temperatures >200 °C. Likewise, according to Preti et al. [34], the total BA content (HIS, SPM, SPD, PUT, PHE, CAD, TYR, ethylamine, methylamine, serotonin, and agmatine) in three varieties of green beans did not change appreciably or even increased after boiling.

3.3. Risk assessment

The mean daily intakes of five seasonings based on the KNHANES database (2021) are shown in Table 3. The daily intake of seasonings ranged from 7.87 to 34.06 g/day, which contributed to a small portion of the total daily food intake. However, a high BA intake could pose a potential risk for the consumer; therefore, calculating the daily intake of seasonings and BA together was imperative to assess the risk of ingesting toxic amounts of BA. In addition, we also calculated the EDI values for cooked seasonings because of the influence of the cooking method on their BA contents.

In the worst-case scenario, the EDI values of different BAs in five types of seasoning before and after cooking were calculated across different age groups. Based on the results (Fig. 3 and Table S3), *Cheonggukjang* had the highest EDI values for each BA among the five types of raw (uncooked) seasoning. This is because of the higher mean daily intake of *Cheonggukjang*, at 34.06 g/day for all consumers, compared to 7.87 g/day for soy sauce, 10.39 g/day for *Gochujang*, 11.45 g/day for *Doenjang*, and 13.52 g/day for *Ssamjang*. People

Table 2
Biogenic amine content (mg/kg) of four fermented seasonings before and after cooking.

Seasonings	TM[1]	TRY	PHE	PUT	CAD	HIS	TYR	SPD	SPM	Total
Cheonggukjang	C	40.83 ± 1.91^{g}	12.45 ± 0.65^{d}	8.44 ± 0.91^{b}	20.42 ± 1.30^{d}	45.68 ± 1.42^{b}	134.57 ± 1.96^{f}	9.10 ± 0.43^{e}	46.76 ± 2.26^{d}	318.24 ± 5.17^{h}
	B	40.63 ± 1.36^{g}	12.91 ± 0.50^{d}	6.83 ± 0.54^{a}	21.70 ± 1.29^{ef}	43.99 ± 2.04^{b}	126.21 ± 1.36^{e}	8.47 ± 0.26^{d}	44.73 ± 3.86^{d}	305.48 ± 7.32^{g}
Soy sauce	C	23.24 ± 0.61^{f}	54.90 ± 0.63^{g}	19.22 ± 0.24^{d}	22.78 ± 0.35^{f}	89.66 ± 1.97^{f}	31.43 ± 1.08^{d}	2.54 ± 0.14^{b}	18.46 ± 0.42^{c}	262.22 ± 1.23^{f}
	B	9.28 ± 0.26^{d}	44.36 ± 0.31^{f}	18.24 ± 0.11^{d}	20.76 ± 0.46^{de}	90.28 ± 0.80^{f}	29.24 ± 0.09^{c}	2.19 ± 0.10^{b}	10.43 ± 0.43^{b}	224.78 ± 1.68^{e}
	SF	11.77 ± 0.30^{e}	22.21 ± 0.24^{e}	12.38 ± 0.11^{c}	11.05 ± 0.14^{c}	73.65 ± 1.77^{c}	1.82 ± 0.09^{a}	*	1.73 ± 0.04^{a}	135.15 ± 2.21^{c}
Doenjang	C	6.64 ± 0.13^{c}	*	35.92 ± 1.71^{e}	*	87.10 ± 0.32^{e}	27.15 ± 0.21^{b}	*	3.78 ± 0.05^{a}	160.59 ± 1.58^{d}
	B	6.15 ± 0.23^{bc}	*	38.45 ± 1.33^{f}	*	80.49 ± 2.39^{d}	30.60 ± 0.97^{cd}	*	3.79 ± 0.08^{a}	159.47 ± 2.29^{d}
Gochujang	C	5.20 ± 0.08^{b}	9.40 ± 0.17^{c}	8.54 ± 0.41^{b}	5.87 ± 0.11^{b}	3.70 ± 0.17^{a}	3.13 ± 0.13^{a}	2.96 ± 0.07^{c}	3.93 ± 0.16^{a}	42.72 ± 0.51^{b}
	B	2.74 ± 0.08^{a}	7.18 ± 0.11^{b}	8.67 ± 0.34^{b}	5.82 ± 0.12^{b}	3.65 ± 0.14^{a}	3.27 ± 0.27^{a}	2.93 ± 0.06^{c}	3.97 ± 0.23^{a}	38.23 ± 0.32^{b}
	SF	1.74 ± 0.11^{a}	2.49 ± 0.03^{a}	7.02 ± 0.03^{a}	1.45 ± 0.04^{a}	3.07 ± 0.03^{a}	2.56 ± 0.10^{a}	2.29 ± 0.04^{b}	3.76 ± 0.07^{a}	24.38 ± 0.20^{a}

All values are shown as mean ± standard error ($n = 3$) from the computation of three measurements.
[1] TM (treatment method): C, control (raw/uncooked); B, boiling; SF, stir-frying.
a–h: Values with different superscripts in the columns are significantly different ($p < 0.05$).
*indicate the concentrations were below the respective limit of quantification (LOQ).
TRY, tryptamine; PHE, 2-phenylethylamine; PUT, putrescine; CAD, cadaverine; HIS, histamine; TYR, tyramine; SPM, spermine; SPD, spermidine.

Table 3
Mean daily intakes (DI, g/day) of five seasonings in various age groups and the mean body weight (bw, kg) of each age group.

Seasonings	Measures	Age groups (years)					
		All	1–12	13–19	20–29	30–64	>64
Cheonggukjang ($n = 129$)	DI	34.06	14.10	12.32	63.86	37.42	30.86
	bw	60.73	42.28	49.49	61.53	65.56	60.06
Soy sauce ($n = 4424$)	DI	7.87	6.23	8.04	9.06	8.15	6.98
	bw	62.34	27.90	59.15	67.58	67.55	61.44
Doenjang ($n = 2067$)	DI	11.45	6.29	9.40	9.13	11.21	14.86
	bw	62.30	27.32	58.42	68.04	67.10	61.10
Gochujang ($n = 3115$)	DI	10.39	6.11	10.09	11.12	11.29	8.96
	bw	63.11	29.10	58.25	67.74	67.70	61.94
Ssamjang ($n = 699$)	DI	13.52	4.35	7.65	13.16	14.36	14.22
	bw	66.12	33.06	64.30	67.90	69.10	61.26

aged between 20 and 29 years had an especially high consumption of *Cheonggukjang*, at 63.86 g/day, with the highest EDI values for the individual BAs also found in this age group, at 1.90×10^{-2} (SPM), 2.61×10^{-2} (PUT), 4.92×10^{-2} (SPD), 6.15×10^{-2} (TRY), 1.28×10^{-1} (HIS), 3.25×10^{-1} (TYR), and 7.25×10^{-1} (total BAs) mg/day/kg bw found in raw *Cheonggukjang*. The highest EDI values for CAD and PHE were found in boiled *Cheonggukjang*, at 3.56×10^{-2} (CAD) and 6.49×10^{-2} (PHE) mg/day/kg bw.

The EDI values of raw soy sauce, boiled soy sauce, and raw *Ssamjang* were mostly higher than the other three seasonings. Although there has been no report on the daily intake limits of other BAs for humans, the NOAEL values of 19 mg/kg bw/day for SPM, 83 mg/kg bw/day for SPD, and 180 mg/kg bw/day for CAD and PUT, were established in Wistar rats [35]. Considering these limits, the EDI values in the current study suggest that the studied BAs do not appear to pose an appreciable human health risk. Conversely, El-Ghareeb et al. [13] determined the EDI values for six types of fish as 5.00×10^{-3} (SPM), 1.1×10^{-2} (SPD), 9.90×10^{-2} (TYR), 1.18×10^{-1} (PUT), 1.46×10^{-1} (CAD), 1.51×10^{-1} (HIS), and 4.93×10^{-1} (total BAs) mg/day/kg bw in adults. Comparatively higher values were found in the current study, except for CAD and PUT. There have been several outbreaks of HIS poisoning worldwide [36], generally associated with fish and fish products, including in Korea [37]. Therefore, the EDI values for the seasonings indicated that besides fish and fish products, the BA contents of seasonings should also be carefully considered in assessing the potential risks of exposure to BAs in the diet.

In order to further assess the potential risks of exposure to BAs through seasoning consumption, the HIS and TYR intakes were calculated by multiplication of seasoning intake (median, 95th percentile [P95], and maximum, respectively) (Table 4) and maximum BA concentration (the worst-case scenario), and the results were compared with the ARfD and NOAEL. As illustrated in Table 5, the highest maximum HIS intake was found in soy sauce after boiling, at 14.37 mg/meal/person, which accounted for 28.74% of the ARfD of HIS (50 mg/meal), followed by raw soy sauce and stir-fried soy sauce. However, when calculating HIS intake using the median and P95 intake, raw *Cheonggukjang* was the dominant source of HIS, at 1.18 and 3.72 mg/meal/person, which was consistent with the EDI results. From these results, although the highest HIS content in *Cheonggukjang* (318.46 mg/kg), soy sauce (148.15 mg/kg), *Doenjang* (123.65 mg/kg), and *Ssamjang* (114.07 mg/kg) exceeded the HIS limit set by the United States Food and Drug Administration (FDA) for

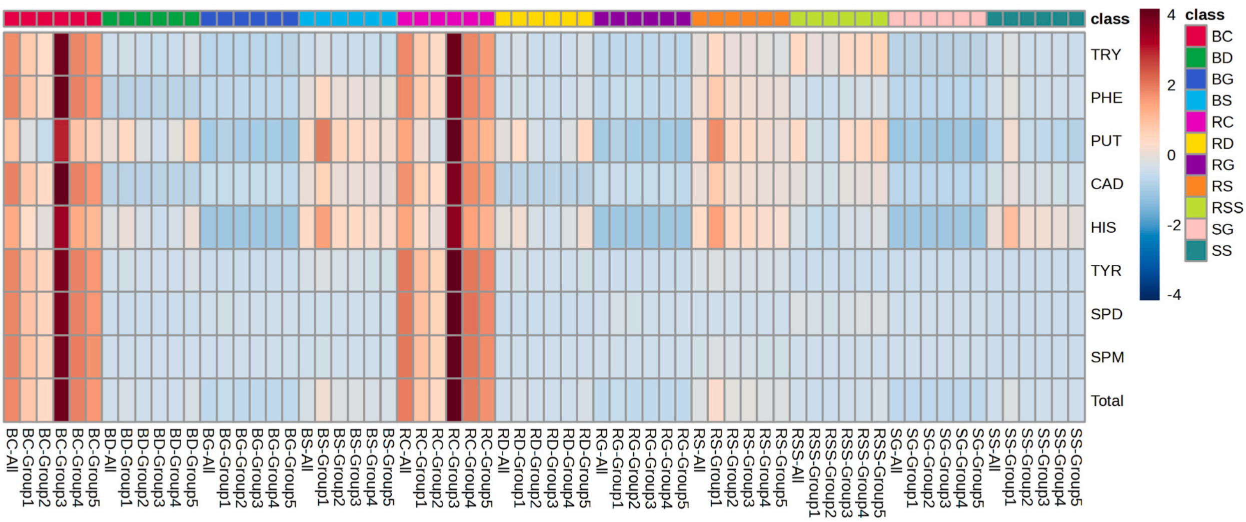

Fig. 3. Heatmap of biogenic amines EDI (mg/day/kg bw) in seasonings before and after cooking for various age groups. BC: boiled *Cheonggukjang*; RC: raw *Cheonggukjang*; BD: boiled *Doenjang*; RD: raw *Doenjang*; BG: boiled *Gochujang*; SG: stir-fried *Gochujang*; RG: raw *Gochujang*; BS: boiled soy sauce; SS: stir-fried soy sauce; RS: raw soy sauce; RSS: raw *Ssamjang*; Group 1: 1−12 years; Group 2: 13−19 years; Group 3: 20−29 years; Group 4: 30−64 years; Group 5: >64 years.

Table 4
Mean, median, P95, and maximum intakes (g/meal) of five seasonings.

Seasonings	Mean	Median	P95	Maximum
Cheonggukjang	11.35	9.57	30.12	46.07
Soy sauce	2.62	1.53	8.29	44.80
Doenjang	3.82	2.96	14.15	55.79
Gochujang	3.46	1.78	12.69	53.10
Ssamjang	4.51	2.17	17.33	43.33

P95, 95th percentile.

Table 5
Median, P95, and maximum intakes (mg/meal/person) of HIS and TYR from five seasonings exposed to different cooking methods.

Seasonings	TM	HIS			TYR		
		Median	P95	Maximum	Median	P95	Maximum
Cheonggukjang	C	1.18	3.72	5.70	3.00	9.42	14.42
	B	1.14	3.59	5.49	2.81	8.84	13.52
Soy sauce	C	0.48	2.64	14.27	0.17	0.95	5.13
	B	0.49	2.66	14.37	0.16	0.88	4.77
	SF	0.40	2.17	11.73	0.01	0.06	0.30
Doenjang	C	0.44	2.10	8.26	0.14	0.67	2.62
	B	0.41	1.94	7.64	0.16	0.75	2.95
Gochujang	C	0.02	0.11	0.44	0.03	0.21	0.86
	B	0.01	0.10	0.44	0.03	0.22	0.90
	SF	0.01	0.09	0.37	0.02	0.17	0.71
Ssamjang	C	0.25	1.97	4.94	0.03	0.23	0.58

P95, 95th percentile; HIS, histamine; TYR, tyramine. TM, treatment method; C, control; B, boiling; SF, stir-frying.

fish products (50 mg/kg) [38], the potential risk of HIS exposure from ingestion of the seasoning alone was limited due to the low intake. Nevertheless, when cooking food with a high HIS content, the seasoning may drive the HIS level beyond a safe level.

Furthermore, it is noteworthy that several types of drugs (e.g., antidepressant drugs and some neuromuscular blocking drugs) can inhibit amino oxidases (monoamine oxidases and diamine oxidases), which metabolize small quantities of BAs in the human gut to physiologically less active forms [5]. Therefore, neurological and psychiatric disorders often treated with monoamine oxidase inhibitor (MAOI) drugs can elevate BA levels because of the inadequate detoxification of BAs. This is particularly relevant for TYR, for which dietary restrictions are often issued to patients taking MAOI medication due to the risk of hypertension [39]. The number of people diagnosed per month with depression in Korea was 312,601 in 2017, and there is an increasing trend in the incidence of depression in the Korean population [40]. Consequently, those prescribed MAOI drugs should also be considered when assessing the risk of exposure to BAs. From a previous report [5], the NOAEL for TYR was 600 mg/meal/person for healthy people, 50 mg/meal/person for patients taking third-generation MAOI drugs, and 6 mg/meal/person for those under treatment with classical MAOI drugs. Based on these data, all seasonings had a safe value for healthy people in terms of TYR intake (mg/meal/person). Nonetheless, among them, raw *Cheonggukjang* had the highest value, with a median of 3.00 mg/meal/person, P95 of 9.42 mg/meal/person, and a maximum of 14.42 mg/meal/person, and the latter two values exceeded the limit (6 mg/meal/person) of TYR intake for people under treatment with classical MAOI drugs, as was also the case with boiled *Cheonggukjang*. In addition, the maximum intakes of TYR from raw soy sauce and boiled soy sauce were 5.13 and 4.77 mg/meal/person, respectively, and both accounted for over 80% of the limit mentioned above. This figure decreased to 0.29 mg/meal/person after stir-frying. Thus, changing the cooking method might reduce the risk of exposure to BAs from soy sauce for people taking classical MAOI drugs. From a former study [14], the median, P95, and maximum exposure to TYR through the consumption of fermented sausage were 3.7, 21.0, and 92.6 mg/meal, respectively; thus, for patients under treatment with MAOI drugs, there was an estimated 38% probability to exceed the safe threshold dose (6 mg/meal).

4. Conclusion

This study monitored BAs in 50 brands of five different Korean seasonings. Except for *Gochujang*, the other types of seasoning showed high contents of BAs, especially *Cheonggukjang* and soy sauce, which had the most TYR and HIS, respectively. During the cooking process, the concentration of BAs decreased dramatically after stir-frying compared to boiling due to the high temperature and direct contact with the hot plate. In a worst-case scenario, the EDI values and HIS intake results showed that the risk of HIS poisoning from seasoning consumption was limited because of the low seasoning intake per meal. Nonetheless, the TYR intake results showed that *Cheonggukjang* should be controlled in daily meals for people taking classical MAOI drugs. These results suggest that the risk of exposure to BAs in these fermented seasonings is negligible for the general population in Korea. However, the risk assessment for some populations indicates that the TYR content in *Cheonggukjang* should be given attention and further studied to provide potential cooking methods for reducing this level.

Author contribution statement

BaoZhu Shi: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

BoKyung Moon: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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