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Quality assessment of fermented soybeans: physicochemical, bioactive compounds and biogenic amines

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Abstract

In this research, a total of 47 samples from five types of fermented soybeans were selected. The physicochemical parameters, biogenic amines (BAs) and bioactive compounds such as isoflavones, total phenolic (TPC) and γ-aminobutyric acid (GABA) were investigated. The results showed that the contents of isoflavones ranged from 598.06 mg/kg to 2725.03 mg/kg in all samples and it varied from 598.96 mg/kg to 2241.67 mg/kg in Semen Sojae Preparatum (SSP) which was higher than other types of fermented soybeans. The maximum variation of GABA content was found in *Aspergillus*-type douchi (AD) samples. The maximum value of TPC in SSP was 4.53 g/kg. For BAs, the indicator of spoilage, 4.26% of fermented soybeans samples may lead to histamine poisoning and 42.55% of samples with total BAs contents exceeding 100 mg/kg may be harmful to human body. It indicated that different types of fermented soybeans had various bioactive compounds and BAs, so different fermented soybeans could be selected according to bioactive substances. This finding could provide comprehensive information of health beneficial compounds and BAs risks of fermented soybeans. It could guide consumers to select healthy, nutritious and safety fermented soybeans.

Keywords: fermented soybeans; isoflavones; γ-aminobutyric acid; total phenolic; biogenic amines.

Practical Application: Monitoring bioactive substances and biogenic amines in fermented soybeans adds value to the entire production chain and increases food safety. Approach to providing guidance for consumers to choose healthier and safer food.

1 Introduction

Soybeans (*Glycine* max (L.) Merri.) are the mature seeds of leguminous plants originated in China and widely cultivated around the world (Park et al., 2019). Soybean product contain essential nutrients and a number of other functional ingredients that are commonly used as dietary supplements due to their health benefits (Rizzo, 2020). Soybeans and its derived foods such as soymilk, sauce, natto, sufu and douchi are popular in the current food market. There are two types of douchi samples based on the taste, one is called salty douchi and the other is a non-salty product named Semen Sojae Preparatum (SSP), also called dandouchi in China, which can be used both as medicine and food (Luo et al., 2021). Natto was fermented with *B. subtilis* that originated in ancient China and evolved into a new form of fermented food after being exported to Japan (Yang et al., 2021). Among them, natto, douchi and SSP were fermented directly from cooked soybean seeds. In general, the production of salty douchi was achieved by two processes: pre-fermentation and post-fermentation. Depending on the microorganisms used in pre-fermentation, salty douchi can be divided into *Aspergillus*-type douchi (AD), *Mucor*-type douchi (MD) and *Bacterial*-type douchi (BD) (Liu et al., 2020). The five types of fermented soybeans were prepared on the basis of a procedure as shown in Figure 1.

The fermentation process changed the physicochemical parameters and enriched the sensory properties of soybeans. These differences in properties are influenced by the composition of the substrate, the choice of starter culture and environmental factors (Qiao et al., 2022). The SSP was naturally fermented by *Artemisia annua* L. (*A. annua*) and *Morus alba* L. (*M. alba*) containing high contents of isoflavones, γ-aminobutyric acid (GABA), oligosaccharides and thrombolytic enzymes. GABA in SSP played a certain role in the treatment of epilepsy, mental disease and Parkinson's disease (Chen et al., 2021). Otherwise, SSP properly had excellent therapeutic and health care efficacy in colds, headache, chest tightness, anorexia, and atrophic gastritis (Chai et al., 2017). The process of soybean fermentation could eliminate the anti-nutritional content and produce a variety of healthy promoting factors. Bioactive compounds in fermented soybeans had been investigated in large number of studies (Jayachandran & Xu, 2019; Liu et al., 2020). It had many health-promoting effects including anti-oxidant, anti-diabetic, anti-cancer, properties and protective effects against cardiovascular disease (Rinaldoni et al., 2014). Xu et al. (2017) found a high GABA level in fermented soybeans, so the fermented soybeans could be used as a supplement of GABA (Xu et al., 2017). Moreover, some researches revealed the ability of conversion isoflavone

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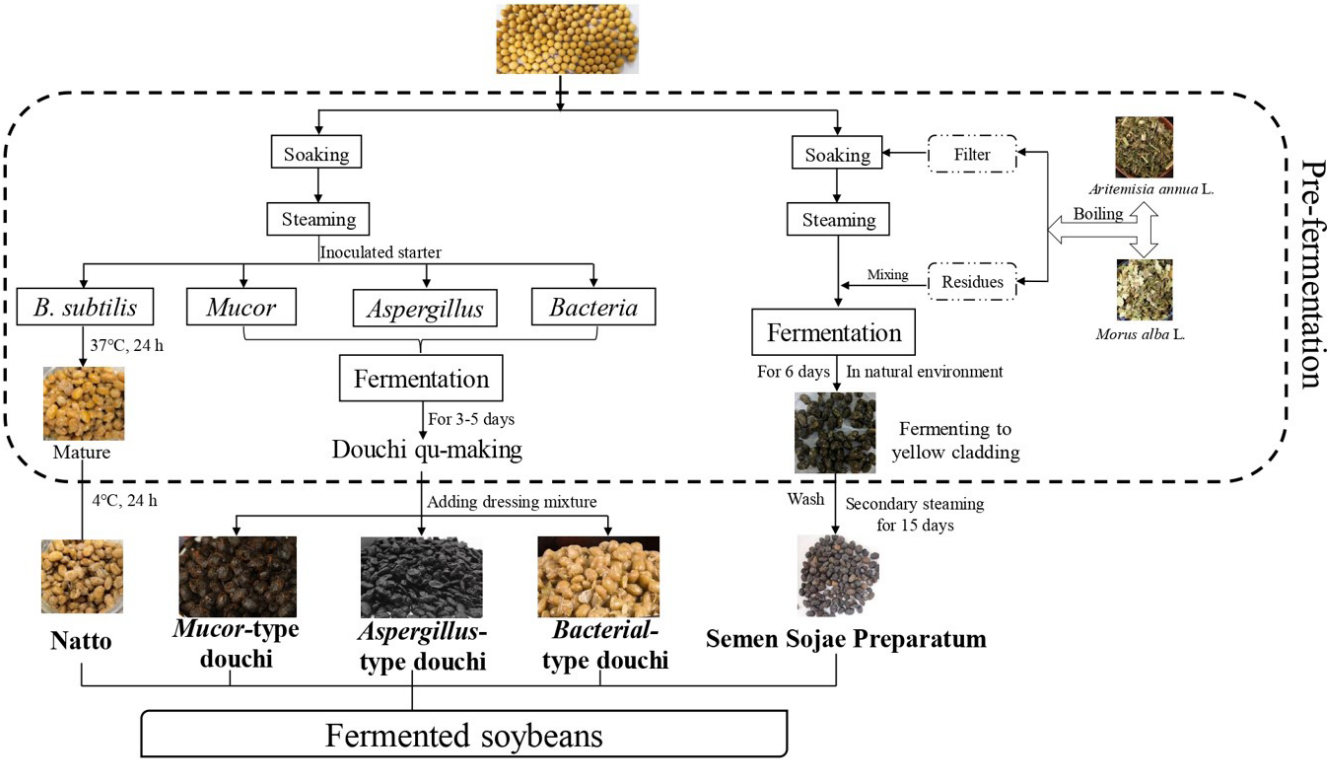

Figure 1. The procedure to prepare five types of fermented soybeans.

glycosides into isoflavone aglycones in the process of soybean fermentation which could enhance the bioavailability and antioxidant properties (Piao & Eun, 2020). Therefore, studying the bioactivity compounds of fermented soybeans was necessary and meaningful.

The fermented soybeans were inoculated various microorganisms and fermented at semi-open environment which could increase the potential safety risk such as biogenic amines (BAs) (Tan et al., 2019). BAs is a class of low wight nitrogen-containing organic compounds which can be found in high proteins food, especially in fermented soybeans. BAs is a precursor of biological macromolecule and plays an essential role in improving human immunity and enhancing vascular activity at low concentration. However, excessive intake of BAs can cause health problems, such as headaches, vomiting, hypotension and palpitations (Li & Lu, 2020). The formation of BAs was influenced by several factors such as raw material quality, pH, temperature, the growth of decarboxylase-positive microorganisms and the availability of free amino acid (Zhang et al., 2022). In our previous work, it showed a correlation between amino acid decarboxylase and BAs content during sufu fermentation (Liu et al., 2022c). There was little information on the contents of bioactive compounds and BAs in SSP. Thus, it is of great significance to understand the bioactive compounds in fermented soybeans and the harmful factors that may be produced during fermentation.

The objective of this work was to assess the contents of multiple bioactive compounds and tracked the BAs content in five types of fermented soybeans. The investigation covered the contents of isoflavones, total phenolic (TPC), GABA, BAs and physicochemical parameters including pH, water content, amino nitrogen and total acids in fermented soybeans. The results obtained in this work could help to deepen the understanding of the bioactive compounds and safety of fermented soybeans. To provide guidance for consumers to choose healthier and safer food.

2 Materials and methods

2.1 Samples collection

A total of 47 fermented soybeans samples were purchased from online markets. Each sample was bought in triplicate. Based on the production process it could be divided into five types: SSP (15 samples), MD (6 samples), AD (12 samples), BD (7 samples) and natto (7 samples). The production areas of these samples were covered a broad range such as Sichuan, Shandong, Beijing, Hebei and Japan. All samples were pulverized and stored at -20 °C for further analysis. Table 1 provided a description of the samples selected.

2.2 Chemical and reagents

The standard of daidzein, daidzin, genistein, genistin, glycitein, glycitin and dansyl chloride were acquired from Yuanye Bio-Technology Co., Ltd (Shanghai, China). The analytical standards of 7 BAs (histamine, tyramine, phenethylamine, tryptamine, cadaverine, putrescine and spermine) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) (purity >

Table 1. Description of fermented soybeans.

Types	Sample name	Produce of origin	n
1	SSP	Anhui, Hebei, Guangxi, Sichuan, Guangdong	15
2	MD	Sichuan, Chongqing	6
3	AD	Jiangxi, Hunan, Guangdong, Beijing, Taiwan	12
4	BD	Guiyang, Yunnan, Shandong	7
5	Natto	Shandong, Japan	7

97%). Chromatographic grade of methanol, ammonium acetate, and acetonitrile were purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Other chemicals were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China).

2.3 Physicochemical parameters determination

The water content was measured by standard gravimetric method at 105 °C (Li et al., 2021b) and the pH value was determined by a pH meter (Li et al., 2021c).

The content of amino nitrogen and total acids contents were measured through the formalin titration method (Zhao et al., 2021). In brief, approximately 5 g of samples were homogenized with 100 mL ultrapure water. After extraction in 70 °C for 10 min, the mixture was filtered. The sample solution (10 mL) was mixed with 50 mL ultrapure water and titrated to pH = 8.2 with 0.05 mol/L NaOH. The volume of consumed NaOH was recorded to calculate the total acids contents. Then, 10 mL formalin solution was added and titrated to pH = 9.2 using 0.05 mol/L NaOH. The volume of total consumed NaOH was taken to determine amino nitrogen.

2.4 Isoflavones determination

Determination of isoflavones contents were using High Performance Liquid Chromatography (HPLC) system and isoflavones were extracted according to the method with slight modifications (Yu et al., 2021). Briefly, the samples (1 g) were dissolved in 30 mL of 90% methanol and incubated in ultrasonic cleaning machine at 60 °C for 1 h. Then the mixture was centrifuged at 4000 rpm for 30 min. The supernatant was filtered through a 0.22 μm filter prior to HPLC assay. An Agilent ZORBAX Eclipse Plus C_{18} column (4.6 mm × 250 mm, 5 μm) was employed for chromatographic separation at 40 °C. The gradient elution program was set with a flow rate of 1 mL/min and the UV detection was performed at 260 nm. The HPLC system was described by Chinese national standard (General Administration of Quality Supervision, Inspection and Quarantine of China, 2011).

2.5 GABA determination

HPLC analysis of GABA was carried out according to a previous method with some modifications (Xu et al., 2015). The GABA extracts were prepared with 1 g of samples from each species and homogenized with 10 mL of 7% acetic acid solution for 1 h. Then the mixture was centrifuged at 10000 rpm for 10 min. 2 mL of supernatant was mixed with 2 mL ethanol left at 4 °C for 2 h to remove the protein and polysaccharides. The mixture was centrifuged at 10000 rpm for 10 min. The supernatant was derivatized with phenyl isothiocyanate reagent and filtered through a 0.22 μm filter paper prior to injection. The HPLC systems were described by Chinese national standard (Ministry of Industry and Information Technology of the People's Republic of China, 2012).

2.6 TPC determination

The TPC was determined by Folin Ciocalteu phenolic according to a method (Rosa & Medina, 2022) with minor modification. A calibration curve was prepared using gallic acid standard solution. 2 g samples were mixed with 20 mL of 70% ethanol and kept in ultrasonic cleaning machine at 40 °C for 30 min. Then the mixture was centrifuged at 4000 rpm for 20 min. The supernatant (1 mL), 2 mL Folin Ciocalteu phenolic and 2 mL 0.2 g/mL Na_2CO_3 was vortexed and adjusted the volume to 25 mL with distilled water. The mixture was incubated for 15 min at 45 °C and measured at an absorbance of 765 nm.

2.7 BAs determination

The content of BAs was analyzed by acid extraction and derived according to a previously research (Liang et al., 2019). Briefly, 1 g of samples were blended with 3 mL of 0.1 mol/L HCl and centrifuged at 7500 rpm for 30 min. 1 mL of supernatant was added, together with 0.2 mL of 2 mol/L NaOH, 0.3 mL saturated Na_2CO_3 solution and 1 mL of dansyl chloride. After blending, the mixture was incubated at 42 °C for 45 min. Then, 0.1 mL of ammonia hydroxide was added and stored at room temperature for 30 min. The mixture was adjusted the volume to 5 mL with acetonitrile and filtered through 0.22 μm filters before HPLC analysis. The HPLC system was described by previously report (Li et al., 2019). The biogenic amine-dansyl adduct was detected at 254 nm wavelength.

2.8 Statistical analysis

All experiments were performed in triplicates and the data was expressed as mean ± standard deviation. The statistical analysis was performed using Origin 2019 software (Origin Lab Co., Ltd; USA). Duncan's multiple range test and One-way ANOVA variance analysis were performed in order to compare significant differences ($p < 0.05$) using SPSS 26.0 for Windows (IBM).

3 Results and discussion

3.1 Physicochemical properties

The physicochemical characteristics of five types fermented soybeans were summarized in Figure 2. The pH value ranged from 5.16 to 6.78, 4.99 to 5.39, 4.83 to 5.78, 5.02 to 7.62, 6.85 to 7.51 in SSP, MD, AD, BD and natto. The water content ranged from 5.58% to 12.54% in SSP, 29.50% to 42.80% in MD, 15.83% to 51.77% in AD, 14.47% to 70.88% in BD and 36.75% to 42.01% in natto. The AD samples presented highest variation of water content which may be due to the different preparation

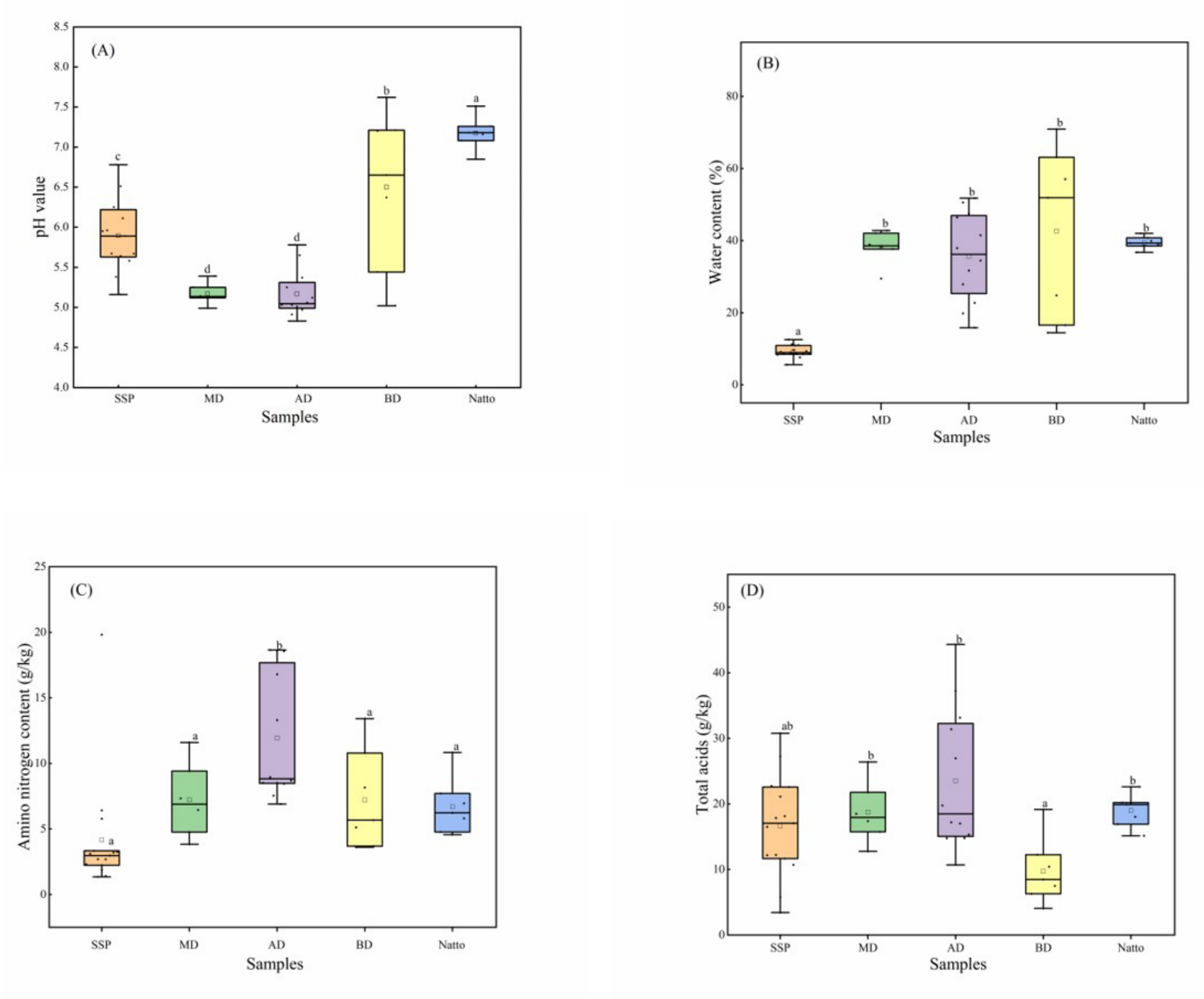

Figure 2. pH value (A), water content (B), nitrogen amino content (C) and total acids contents (D) for multiple fermented soybeans.

methods and dressing mixture. The SSP showed a minimum variation could be attributed to the SSP were dried at the end of fermentation. There was no significant difference in the mean water content of MD and AD. This finding was consistent with earlier observations which studied the changes of physical characteristics of rapid fermented douchi with different starter strain (*Mucor* and *Aspergillus*) (Tan et al., 2019).

Amino nitrogen content was a key factor for indicating fermentation maturity and evaluating the quality of food. The mean content of amino nitrogen of AD were significantly higher than other types of fermented soybeans. The content of amino nitrogen in AD varied from 6.90 g/kg to 18.65 g/kg and it varied from 4.56 to 10.83 g/kg in natto. A previous study determined the content of amino nitrogen in natto fermented by different strains and conditions. The results showed that the amino nitrogen content varied from 2.82 to 7.06 g/kg, the content could be affected by different strains and fermentation conditions (Lan et al., 2020). The total acids contents ranged from 3.42 g/kg to 30.78 g/kg in SSP, varied from 12.76 g/kg to 26.39 g/kg in MD, ranged from 10.69 g/kg to 44.31 g/kg in AD, ranged from 4.08 g/kg to 19.15 g/kg in BD and ranged from 15.12 g/kg to 22.61 g/kg in natto. The total acids contents in AD were higher than that other four types which can be due to the excessive proteolysis to produce small molecular substances that increase the total acids contents (Li et al., 2021c).

3.2 Isoflavones

Isoflavones had been reported to have beneficial effects such as estrogenic, antioxidative, anticarcinogenic and antiosteoporosis activity. Previous research had also mentioned that in soybean and non-fermentation soybean products exhibited 12 kinds of isoflavones which mainly exist in the form of glycosides (daidzin, genistin, glycitin), accounting for 97%-98% (Tyug et al., 2010). During the fermentation process, the existing form of isoflavones contents changed significantly, furthermore native forms of isoflavones were hydrolyzed by β-glucosidase produced by microorganisms to absorbable aglycones (daidzein, genistein, glycitein) (Liu et al., 2022a).

The isoflavones contents of fermented soybeans were shown in Figure 3. The contents of isoflavones in different types of fermented soybeans exhibited a significant difference. It was clearly observed that daidzein, daidzin, genistein and genistin

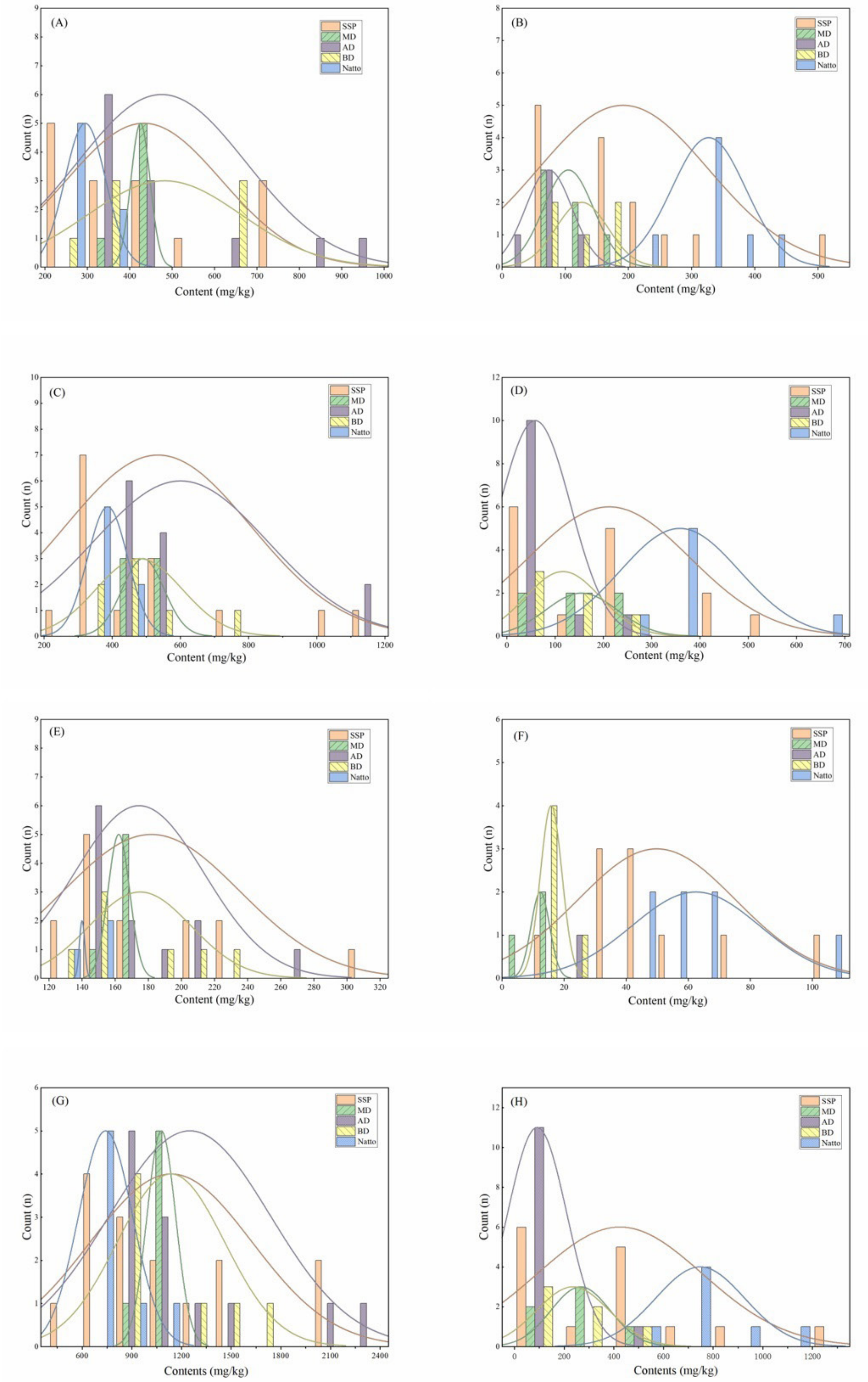

Figure 3. Isoflavones contents distribution diagram in different fermented soybeans. A: Daidzein content; B: Daidzin content; C: Genistein content; D: Genistin content; E: Glycitein content; F: Glycitin content; G: Total aglycones contents; H: Total glycosides contents.

were the main isoflavones. As shown in Figure 3A, the content of daidzein was highest in AD (909.38 mg/kg), followed by SSP (748.70 mg/kg). The content of daidzin was depicted in Figure 3B. The lowest mean content of daidzin was found in AD (73.97 mg/kg) and SSP showed maximum variation ranged from ND to 541.44 mg/kg. The daidzin content of BD ranged from ND to 183.40 mg/kg. The distribution diagram of genistein was shown in Figure 3C. The minimum variation (161.89 mg/kg) of genistein in MD varied from 411.63 mg/kg to 573.52 mg/kg. The highest variation (880.82 mg/kg) of genistein in SSP ranged from 297.26 mg/kg to 1178.08 mg/kg, the natto samples were ranged from 335.79 mg/kg to 490.37 mg/kg, among them two AD samples contained more than 1000 mg/kg genistein. Figure 3D depicted the distribution of genistin and natto had the highest genistin content (623.77 mg/kg).

The content of glycitein was shown in Figure 3E. It was observed that glycitein content varied significantly from ND to 316.09 mg/kg in natto, followed by AD and BD samples, with the highest content of glycitein was 269.41 mg/kg and 220.48 mg/kg. Glycitein was detected in three natto samples. Glycitin, as the least content of the six isoflavones in fermented soybeans samples, was detected with 21.80 mg/kg in only one AD sample. However, glycitin was found in all natto samples. The content of isoflavones varied from 598.96 mg/kg to 2241.67 mg/kg in SSP which was higher than other types of fermented soybeans and the mean content of aglycone was higher than glycosides in SSP. The results could be due to the *Artemisia annua* L. and *Morus alba* L. promoted the transformation of glycosides into aglycone and increase the aglycone content in SSP (Li et al., 2021a). The mean content of total isoflavones was highest in SSP (1562.11 mg/kg), followed by natto (1489.34 mg/kg). However, the mean content of total aglycones in SSP was 1137.77 mg/kg which was higher than that in natto. The contents of total aglycones varied from 523.20 mg/kg to 2285.02 mg/kg and total glycosides contents ranged from ND to 1223.72 mg/kg in all fermented soybeans. The total aglycones contents were significantly higher than the total glycosides contents. It is well known that SSP had abundant components which could be able to use in traditional Chinese medicine for curing disease and protecting humans' health. Hence, the fermented soybeans had been shown to exert a higher biological activity especially SSP contained high isoflavones content (Liu et al., 2022b).

3.3 GABA

GABA is a non-protein amino acid which is synthesized by decarboxylation of glutamic acid catalyzed by decarboxylase. Some researches had revealed that GABA was an inhibitory neurotransmitter in the brain and spinal cord of mammals. It could alleviate pain and anxiety, regulate blood pressure and also prevent diabetes effectively (Tiansawang et al., 2016). Figure 4 presented the content of GABA in the all samples. The content of GABA in the same type of samples existed a significant difference. The SSP samples presented minimum variations in GABA content ranged from 32.41 mg/kg to 357.15 mg/kg. The GABA content of MD samples ranged from 100.65 mg/kg to 510.85 mg/kg and the BD samples ranged from 23.09 mg/kg to 612.17 mg/kg. The maximum variation of GABA content was observed in AD samples ranged from 93.60 mg/kg to 1190.31 mg/kg. GABA was detected in only one natto sample with a content of 24.25 mg/kg. A previous study determined the GABA content in five commercial douchi. The result showed that the content of GABA in douchi ranged from 1.75 mg/g to 5.25 mg/g and demonstrated that fermentation could increase the GABA content in a large scale (Xu et al., 2017). The AD samples had slightly more GABA content compared to other types of fermented soybeans. The differences in the sources of soybean raw materials and different fermentation conditions may have led to the variation in GABA content. Therefore, it is necessary to further study how to control the fermentation conditions to promote the produce of GABA.

3.4 TPC

The distribution of TPC in different fermented soybeans represented in Figure 5. It was observed that the TPC value exhibited a significant difference in five types of fermented

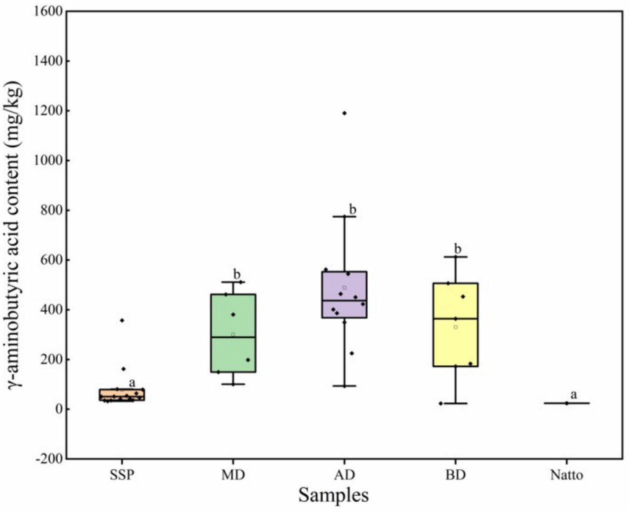

Figure 4. GABA content (g/kg) in different fermented soybeans.

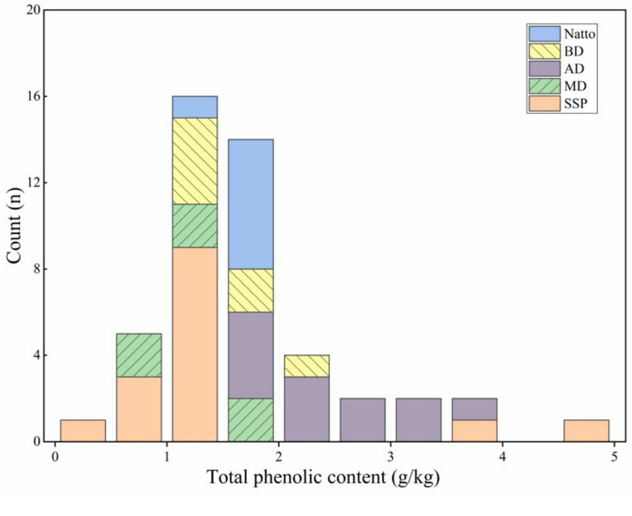

Figure 5. The distribution graph of TPC content (g/kg) in different fermented soybeans.

soybeans. The SSP presented a highest variation in TPC value, ranging from 0.42 g/kg to 4.53 g/kg. The maximum TPC value of was found in SSP which is ten times of the minimum. The mean content of AD samples was significantly higher than the other types. The BD samples ranged from 1.05 g/kg to 2.03 g/kg and the MD samples ranged from 0.63 g/kg to 1.76 g/kg. The minimum variation was presented in natto (0.47 g/kg), ranging from 1.41 g/kg to 1.88 g/kg. Overall, there were 30 samples with a TPC value between 1.0 g/kg and 2.0 g/kg. Xu et al. (2015) studied the correlation between bioactive substances and overall antioxidant capacities of commercial fermentation soybean food. The result showed that TPC value in entire douchi samples was higher than other types of fermented soybean products (Xu et al., 2015).

Phenolic substances were often considered as the most important bioactive components in soybeans food which could contribute to improve the overall antioxidant capacity in soybeans and related food products. Previous research demonstrated the antioxidant activity of food was significant related to the high TPC value. A previous study reported that adding citrus peel can increase the content of TPC, aglycone-form isoflavone, organic acid and antioxidant during moromi fermentation of soy sauce (Peng et al., 2018). Hence, the TPC value in fermented soybeans can be improved by changing the fermentation conditions.

3.5 BAs

High content of BAs in foods constituted a potential public health concern and received extensive attention. The FDA had shown that exposed to histamine content exceeding 50 mg/kg in aquatic products and took greater than 100 mg/kg total BAs contents in food had negative health effects (US Food and Drug Administration, 2011). The EFSA imposed maximum limits of histamine content in food at 100 mg/kg (European Food Safety Authority, 2011). The level of BAs in five categories of different fermented soybeans were shown in Table 2. It was observed that BAs in various types of samples exhibited a significant difference.

Table 2. Biogenic amines content (mg/kg) for fermented soybeans.

Samples		Tryptamine	Phenethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermine	Total BAs
SSP	1	ND	ND	9.61 ± 2.06^{f}	ND	ND	4.01 ± 0.41^{g}	24.64 ± 3.01^{e}	38.26
	2	9.55 ± 0.10^{cd}	ND	9.87 ± 1.08^{f}	ND	ND	ND	30.71 ± 5.11^{d}	50.13
	3	ND	ND	9.23 ± 0.24^{f}	ND	ND	ND	28.93 ± 0.54^{d}	38.16
	4	18.53 ± 0.67^{c}	ND	181.67 ± 30.39^{c}	230.24 ± 35.22^{d}	12.99 ± 0.54^{c}	159.86 ± 13.59^{c}	9.3 ± 1.60^{h}	612.59
	5	ND	ND	9.21 ± 0.79^{f}	ND	ND	ND	38.43 ± 5.17^{c}	47.64
	6	240.07 ± 33.72^{a}	315.03 ± 59.54^{b}	362.01 ± 40.08^{a}	563.92 ± 29.22^{a}	32.61 ± 3.08^{b}	664.53 ± 12.82^{a}	ND	2178.17
	7	ND	ND	2.49 ± 0.70^{f}	ND	ND	ND	11.55 ± 0.72^{gh}	14.04
	8	ND	2.01 ± 0.01^{c}	52.04 ± 1.69^{e}	18.33 ± 0.53^{e}	ND	69.97 ± 2.16^{f}	50.16 ± 3.11^{a}	192.51
	9	ND	291.99 ± 17.83^{b}	151.4 ± 6.17^{d}	412.95 ± 23.08^{c}	34.44 ± 1.13^{b}	631.67 ± 53.16^{b}	ND	1522.45
	10	ND	ND	56.09 ± 0.83^{e}	20.29 ± 0.14^{e}	ND	58.93 ± 1.61^{f}	45.65 ± 1.15^{b}	180.96
	11	ND	ND	9.08 ± 0.13^{f}	4.14 ± 0.62^{e}	ND	11.74 ± 0.25^{g}	16.14 ± 0.53^{f}	41.10
	12	ND	ND	6.14 ± 2.26^{f}	ND	ND	ND	26.54 ± 2.39^{de}	32.68
	13	ND	ND	12.77 ± 0.91^{f}	9.76 ± 0.06^{e}	ND	ND	29.38 ± 1.14^{e}	51.91
	14	ND	ND	22.33 ± 1.45^{f}	9.81 ± 2.09^{e}	ND	4.37 ± 0.25^{g}	13.88 ± 1.39^{fg}	50.39
	15	149.42 ± 8.31^{b}	486.45 ± 31.59^{a}	292.21 ± 11.75^{b}	466.73 ± 21.14^{b}	81.25 ± 8.82^{a}	122.6 ± 26.36^{e}	ND	1598.66
MD	1	ND	6.41 ± 0.07^{a}	15.88 ± 1.33^{ab}	3.60 ± 0.64^{a}	ND	2.28 ± 0.10^{a}	ND	28.17
	2	ND	ND	16.60 ± 0.15^{a}	ND	ND	ND	2.54 ± 0.01^{c}	19.14
	3	ND	ND	13.77 ± 0.05^{c}	2.65 ± 0.14^{b}	ND	ND	ND	16.42
	4	ND	ND	15.83 ± 0.10^{ab}	3.52 ± 0.21^{a}	ND	ND	2.04 ± 0.02^{d}	21.39
	5	ND	ND	16.39 ± 0.15^{a}	2.37 ± 0.35^{b}	ND	ND	3.28 ± 0.03^{b}	22.04
	6	7.32 ± 0.08^{a}	ND	14.86 ± 0.54^{b}	ND	ND	ND	10.74 ± 0.11^{a}	32.92
AD	1	12.49 ± 0.34^{c}	36.41 ± 1.41^{c}	21.18 ± 1.54^{cd}	183.02 ± 9.64^{a}	58.07 ± 4.88^{a}	438.82 ± 25.42^{a}	ND	749.99
	2	49.95 ± 0.77^{a}	142.74 ± 2.18^{a}	137.48 ± 10.36^{a}	14.59 ± 0.86^{c}	23.19 ± 0.84^{cd}	332.75 ± 6.66^{b}	ND	700.70
	3	ND	7.24 ± 0.05^{f}	17.13 ± 0.64^{d}	1.66 ± 0.01^{d}	24.76 ± 1.09^{c}	63.18 ± 0.89^{g}	2.30 ± 0.07^{a}	116.27
	4	ND	12.94 ± 1.12^{e}	25.55 ± 1.48^{c}	4.26 ± 0.12^{d}	28.49 ± 0.22^{b}	119.83 ± 0.29^{d}	ND	191.07
	5	ND	14.30 ± 2.33^{e}	21.26 ± 0.23^{cd}	6.97 ± 0.12^{d}	22.03 ± 0.60^{cd}	102.99 ± 9.07^{ef}	ND	167.55
	6	9.81 ± 0.03^{d}	10.65 ± 0.75^{ef}	20.38 ± 0.49^{cd}	3.39 ± 0.25^{d}	21.00 ± 0.44^{de}	89.66 ± 6.40^{f}	ND	154.89
	7	5.45 ± 0.04^{f}	12.23 ± 1.06^{f}	16.98 ± 0.98^{e}	2.82 ± 0.02^{d}	25.36 ± 0.84^{c}	106.05 ± 3.79^{de}	ND	168.89
	8	13.49 ± 0.19^{c}	11.27 ± 0.12^{ef}	15.6 ± 1.32^{de}	ND	18.34 ± 3.84^{e}	41.71 ± 0.37^{h}	ND	100.41
	9	21.99 ± 0.07^{b}	107.57 ± 0.93^{b}	41.27 ± 0.67^{b}	148.68 ± 8.49^{b}	14.68 ± 0.17^{f}	142.53 ± 3.03^{c}	ND	476.72
	10	6.07 ± 0.49^{e}	ND	8.68 ± 0.08^{e}	2.02 ± 0.17^{d}	ND	4.26 ± 0.03^{i}	2.28 ± 0.01^{a}	23.31
	11	8.37 ± 0.20^{d}	30.30 ± 1.31^{d}	22.24 ± 0.61^{cd}	5.58 ± 0.72^{d}	9.06 ± 0.09^{g}	93.8 ± 0.19^{ef}	ND	169.35
	12	ND	ND	14.98 ± 0.35^{de}	4.08 ± 0.41^{d}	ND	ND	ND	19.06

Numbers are the means of three replicates per samples ± standard deviation. Different superscript letters in the same column in the same group represent significant statistical differences at $P < 0.05$. ND: not detected.

Table 2. Continued...

Samples		Tryptamine	Phenethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermine	Total BAs
BD	1	33.1 ± 0.58^{d}	9.98 ± 0.28^{b}	10.54 ± 0.17^{c}	8.82 ± 0.26^{a}	ND	12.88 ± 2.34^{d}	41.55 ± 8.23^{a}	116.87
	2	12.45 ± 0.05^{e}	6.69 ± 0.63^{e}	8.83 ± 2.14^{c}	5.94 ± 0.41^{c}	ND	1.06 ± 1.07^{f}	16.61 ± 0.64^{b}	51.58
	3	37.10 ± 0.85^{c}	6.99 ± 0.09^{e}	14.11 ± 1.35^{b}	1.85 ± 0.03^{f}	ND	18.85 ± 0.05^{c}	16.68 ± 0.05^{b}	95.58
	4	60.29 ± 0.53^{a}	15.46 ± 0.11^{a}	10.3 ± 0.15^{c}	4.25 ± 0.48^{d}	3.71 ± 0.01^{b}	75.05 ± 3.55^{a}	17.14 ± 0.39^{b}	186.20
	5	47.67 ± 2.15^{b}	7.72 ± 0.02^{d}	10.73 ± 0.22^{c}	7.46 ± 0.95^{b}	ND	9.90 ± 0.06^{e}	17.94 ± 0.66^{b}	101.42
	6	47.81 ± 0.24^{b}	8.81 ± 0.10^{c}	3.33 ± 0.58^{d}	3.02 ± 0.01^{e}	ND	45.29 ± 0.02^{b}	14.76 ± 0.37^{b}	123.02
	7	7.62 ± 0.14^{f}	ND	18.95 ± 1.49^{a}	ND	7.8 ± 0.17^{a}	ND	2.68 ± 0.04^{c}	37.05
Natto	1	ND	ND	ND	ND	ND	ND	14.56 ± 2.63^{de}	14.56
	2	ND	ND	ND	ND	ND	ND	16.37 ± 1.29^{cd}	16.37
	3	ND	ND	5.55 ± 0.07^{b}	ND	ND	10.18 ± 0.04^{a}	20.01 ± 0.26^{b}	35.74
	4	ND	ND	4.99 ± 0.07^{c}	ND	ND	8.41 ± 0.34^{b}	10.92 ± 0.29^{f}	24.32
	5	ND	ND	ND	ND	ND	ND	12.71 ± 0.99^{ef}	12.71
	6	ND	ND	ND	ND	ND	ND	18.54 ± 1.34^{bc}	18.54
	7	9 ± 0.01^{a}	ND	6.79 ± 0.07^{a}	6.75 ± 0.02^{a}	ND	ND	29.77 ± 0.18^{a}	52.31

Numbers are the means of three replicates per samples ± standard deviation. Different superscript letters in the same column in the same group represent significant statistical differences at $P < 0.05$. ND: not detected.

The content of BAs in same type of fermented soybeans were significantly different.

The maximum variation of BAs content was observed in SSP samples ranged from 14.04 mg/kg to 2178.17 mg/kg. The main BAs in SSP were putrescine, cadaverine and tyramine and spermine. Histamine was detected in only four SSP samples. There existed three SSP samples contained more than 1000 mg/kg BAs which were harmful to human health (Motaghifar et al., 2021). The highest contents of total BAs found in SSP could be due to the fact that fermentation of SSP was a traditional natural process and the changes in the process were uncontrollable (Guo et al., 2018). Hence, it is better to investigate the starter microbial to reduce the content of BAs. The MD samples did not contain histamine and the dominant BAs were putrescine (13.77-16.60 mg/kg). The total BAs contents in MD samples ranged from 16.42 mg/kg to 32.92 mg/kg which was below the limit value of 100 mg/kg. The BAs levels of AD samples ranged from 19.06 mg/kg to 749.99 mg/kg. Tyramine was the main BAs in AD samples, followed by histamine and putrescine. The spermine was detected in only two samples. The main BAs in BD was tryptamine and the total BAs contents varied from 37.05 mg/kg to 186.20 mg/kg. Histamine and phenylethylamine were not detected in all natto samples and the predominate BAs was spermine.

The total BAs contents of all MD and natto samples did not exceed the limit value of 100 mg/kg. The mean content of total BAs in SSP is 443.31 mg/kg which was higher than other types of fermented soybeans. The mean contents of total BAs in MD (23.35 mg/kg) were lower than other types of fermented soybeans. These results may be due to the adding of alcoholic beverage in the post-fermentation which has a certain inhabited the growth of microorganisms. In this study the fermented soybeans accumulated high total BAs contents. As the most toxic BAs, 4.26% of fermented soybeans may cause histamine toxicity. 42.55% of samples with total BAs contents exceeding 100 mg/kg may be harmful to human body. High levels of BAs may be result in the use of soybeans, inappropriate fermentation conditions and contamination during food processing and storage (Triki et al., 2018). Consequently, BAs content could be used for an indicator of food spoilage. It can provide some useful information for freshness and sanitary quality of fermented soybeans.

4 Conclusion

This work was to determine the physicochemical characters, isoflavones, GABA, TPC and 7 BAs contents in five types of fermented soybeans. The results showed that the AD samples were rich in GABA and TPC. The contents of isoflavones in the form of aglycone were higher than the form of glycosides in all samples. As a traditional medicine and food, SSP was rich in isoflavones which was higher than other types of fermented soybeans. Fermented soybeans may be an excellent resource with numerous bioactive compounds and health-promoting effects. But the consumption of fermented soybeans containing high amounts of BAs may result in healthy risks on body. Hence, further research is needed to control the formation of BAs. The results in the present study provided useful information for understanding the quality of fermented soybeans and will be available for consumers to choose suitable and nutritious fermented soybeans.

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