Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

Isolation and identification of tyramine-producing bacteria and their biogenic amines formation during fermentation of sufu

Yuwei Li, Tingting Yan, Lijun Yin, Yongqiang Cheng, Xin Jia*

College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China

ARTICLE INFO

Original paper

Article history:
Received: November 11, 2021
Accepted: December 20, 2021
Published: January 30, 2022

Keywords:
Biogenic amines; Fermented food; Tyramine-producing bacteria; *Enterococcus* spp.; *Pediococcus* spp.

ABSTRACT

The Chinese traditional soybean product, sufu, is getting increasingly popular in Asia for its special taste and rich nutrition. In this paper**,** physicochemical properties of sufu during fermentation, isolation and characterization of BA-producing bacteria, as well as their decarboxylase activities were thoroughly investigated. Tyramine, putrescine, phenylethylamine and cadaverine were the main BA in sufu fermentation. Tyramine level increased drastically to reach 513.72 mg/kg during sufu ripening, posing potential health risks. During sufu fermentation, there was a positive correlation between amino nitrogen and BA, yet no significant correlation was found between BAs with pH and total acidity. Additionally, 23 strains of tyramine- and phenylethylamine-producing bacteria harboring the TDC gene, including *Enterococcus faecalis* 45, *Enterococcus faecium* 36, *Pediococcus acidilactici* 310 and *Pediococcus pentosaceus* 27 that were responsible for tyramine production in sufu have been characterized. The current study provides insights into understanding the production of tyramine in sufu by microorganisms, which laid the groundwork for controlling biogenic amine production in fermented soybean products.

DOI: http://dx.doi.org/10.14715/cmb/2022.68.1.11

Introduction

Biogenic amines (BAs) are prevalent in foods and most likely occur in fish, cheeses, fermented vegetables and soybean products, beer and wine(1). The most pervasive BA in foods is histamine, putrescine, cadaverine, tyramine, tryptamine, phenylethylamine, spermine and spermidine. Biogenic amines are important from a hygienic point of view as they have been implicated as causative agents in several food poisoning episodes. Consumption of food containing BA is responsible for several types of food-born disease, including scombroid poisoning, also called histamine fish poisoning, and allergy-like form of food poisoning, and cheese reaction caused by tyramine toxicity. BA has also been investigated as possible mutagenic precursors since some amines may be nitrosated or act as precursors for other compounds capable of forming nitrosamines that are carcinogenic to various species of animals, therefore posing potential health threats to humans (2). Histamine and tyramine are the most potent biologically active molecules and can exert many responses within the body (3, 4). Additionally, phenylethylamine has also been proposed as an initiator of hypertension, owing to a similar mechanism as tyramine (European Food Safety Authority (EFSA) 2011). Although not all BA are equally toxic, the presence of specific BA may affect the toxicity of histamine, suggesting the intricate reactivities among BA as well as increasing risk of having multiple amines presence in foods (European Food Safety Authority (EFSA) 2011)(5).

The fermented soybean is well known in China for its advantage of replenishing vitamin B12 and accelerating the absorption of mineral sustains (6). Thus, fermented soybean foods have been popular in Asia for more than one thousand years and are becoming increasingly popular in Western countries (7). Sufu or furu, a traditional fermented soybean food originating in China, has been widely consumed as an appetizer for centuries. In general, four steps are involved in preparing northern style mold-fermented sufu: (1) making tofu, (2) overgrown with a pure culture inoculum of *Actinomucor elegans* to prepare pehtze or maopi, (3) salting to reach the salt concentration of 14% (w/w) of wet weight in pehtze, (4) ripening in dressing mixture for 45 to 90 days (8, 9). Depending on the

*Corresponding author. E-mail: xinjia@cau.edu.cn

choice of dressing mixtures, white, red and grey sufu can be prepared. Studies have shown that as high as 1730 mg/kg tyramine, 730 mg/kg histamine, 463.45 mg/kg tryptamine and 341.03 mg/kg phenylethylamine was detected in commercial sufu (8, 10-13). The occurrence of BA in sufu is caused by bacterial contamination during pre-fermentation (9). Amino acid decarboxylase activities have been identified in certain *Enterobacteriaceae*, *Lactobacillus* and *Pseudomonas* species which commonly exist in sufu (14, 15).

The quality criteria with respect to the presence of histamine and other BA vary from food to food, as the existence of potential effectors dramatically influence the toxic thresholds of histamine in foods (European Food Safety Authority (EFSA) 2011). These effectors include metabolic capabilities of the microorganism presented and conditions of spoilage, ageing or ripening. Other risk factors, such as alcohol and gastrointestinal disease in individuals may also play an essential role in determining the toxic threshold for BA. In general, an intake of over 40 mg BA per meal is considered potentially harmful, and over 1000 mg is supposed to be elicited toxically. “Good Manufacturing Practice” indicates the presence of more than 100 μg/mg, 800 μg/mg, and 30 μg/mg of histamine, tyramine and phenylethylamine, respectively, or a total of more than 200 μg/mg BA in foods or food products are considered unsafe (European Food Safety Authority (EFSA) 2011)(5).

Not only does fermentation microbiota plays a significant role in the development of organoleptic characteristics, but it is also responsible for the accumulation of undesired substances, such as BA. Two strains of *Bacillus subtilis* isolated from 22 types of sufu showed the initial evidence of histamine-producing bacteria in sufu (10). Recently, *Bacillus tropicus*, *Bacillus cereus* and *Bacillus velezensis* have also been demonstrated to produce histamine in sufu (16). *Kurthia* genus bacterium was found to play essential roles in histamine formation during sufu production (16). *Pseudomonas* genus bacteria was found to be correlated with histamine and cadaverine production in sufu (15). To date, little information is known concerning the isolation method and characterization of tyramine-producing bacteria in sufu. In this study, levels of BA at different stages during white sufu production have been analyzed. 23 strains of tyramine-producing bacteria in white sufu have been isolated and characterized; their ability to produce tyramine in sufu has also been investigated. This work provided insights into the understanding mechanism of tyramine production sufu, which laid the groundwork for regulating BA in fermented soybean foods.

Materials and methods

Chemicals and reagents

Biogenic amines standards (tryptamine (purity=98%), phenylethylamine (purity=99%), cadaverine dihydrochloride (purity=98%), putrescine dihydrochloride (purity≥98%), tyramine (purity=98%), histamine (purity=96%), spermidine (purity=97%) and spermine (purity=98%)) and dansyl chloride (Dns-Cl) were purchased from Aladdin (Shanghai, China). HPLC grade acetonitrile and acetone were obtained from Merck (Damstadt, Germany). All chemicals and solvents used were of analytical and chromatographic grade.

Sampling

Sufu samples were taken at a sufu factory located in Beijing, China, at different stages of white sufu production: tofu, pehtze, salted pehtze during pre-fermentation and weekly samples during the ripening stage. Tofu or sufu products from each stage were sampled in triplicates.

Determination of biogenic amine

Sample preparation and derivatization

The Sufu sample (2.5 g) was homogenized in 15 mL of 0.1 M HCl; the mixture was centrifuged at 15800 ×*g* for 20 min before passing through a 0.22 μm syringe filter. Derivatization of BA before HPLC analysis was carried out based on a previously published method by Eerola et al. (1993). An aliquot of a 1 mL sample was neutralized with 200 μL of 2M NaOH solution and 300 μL of saturated $NaHCO_3$ solution. Two mL of derivatization agent dansyl chloride (10 mg/mL in acetone) was then added to the neutralized mixture, and the reaction mixture was incubated in the dark at 40 °C for 45 min. The unreacted dansyl chloride was quenched using 100 μL ammonium hydroxide, the volume of the mixture

was then adjusted to 5 mL with acetonitrile. The sample mixture was filtered through a 0.22 μm syringe filter prior to HPLC analysis.

Apparatus and HPLC conditions

BA concentrations in white sufu samples were measured based on a previously published HPLC method (11). Briefly, an Agilent 1260 high-performance liquid chromatography system consisting of a quaternary pump and a photodiode array absorbance detector (Agilent G1315C) was used for BA analysis. Separations of BA derivatives were achieved using a Capcell Pak C18 column (5 mm, 4.6 mm I.D. 250 mm; Shiseido Co., Japan). Gradient elution with deionized (DI) water (solvent A) and acetonitrile (solvent B) were set as follows: 0-1min, 65% B; 1-15min, 90% B; 15-25min, 90% B; 25-30min, 65% B. The injection volume was set at 20 μL, and the flow rate was 1.0 mL/min. The wavelength of the detector was set at 254 nm. HPLC analyses were performed in triplicate. Data collection and analysis were performed using Chemstation software (Agilent, Germany).

Physicochemical and microbiological measurements

pH, amino nitrogen and microbiological levels were measured throughout the sufu fermentation. Sufu sample (3.0 g) taken at each fermentation stage was homogenized in 27 mL DI water using a high-speed blender (T25-basic, IKA, Germany) at 11,000 rpm for 2 min, and the pH of the suspension was measured by a pH meter (METTLER TOLEDO, China). Amino nitrogen content was determined using the formalin titration method described by Qiu et al. (2018). The microbiological content of sufu was measured using 10 g Sufu samples mixed thoroughly with 90 mL of sterile phosphate buffer. The mixture was serially diluted (10^{-1} to 10^{-7}) with sterile phosphate buffer. Diluted samples were plated in duplicates on Plate Count Agar (PCA) for growth of total count of mesophilic aerobic bacteria (TMAB), and Man Rogosa and Sharp (MRS) supplemented with 0.1% natamycin (Macklin, Shanghai, China) for Lactic Acid Bacteria (LAB). Plates were incubated at 37 °C for 48h ± 2h. Counts were expressed as colony-forming units per gram of sample (CFU/g).

Isolation and characterization of BA-producing bacteria in sufu

Screening for BA-producing bacteria

From each countable MRS agar plate, five to seven isolated colonies were picked systematically and purified by successive streaking. Pure cultures were stored in 50% (v/v) glycerol at -80 °C. Initial screening for potential BA-producing bacteria was carried out based on a method published by Coton et al. (17). Sterile 96-well polystyrene microtiter plates containing 100 μL of liquid decarboxylase medium (DM) (pH 5.3) with 0.1% (w/v) precursor amino acids L-Tryptophan, L-Phenylalanine, L-Lysine, L-Ornithine, L-Histidine, L-Tyrosine, and L-Arginine was inoculated with 30 μL culture of the isolated strain in MRS broth (described above). The mixture was then topped with 70 μL of sterilized liquid paraffin. Sterile DM broth was used as a control. All assays were performed in duplicate. Microtiter plates were incubated at 37 °C for 48 h. The purple color indicates the presence of BA-producing bacteria.

To investigate the ability for selected bacteria to produce BA, an 100 μL aliquot of selected strain was inoculated in 4.9 mL of MRS broth (pH 5.3) supplemented with 0.005% (w/v) of pyridoxal-5-phosphate, 0.1% (w/v) of each precursor amino acid and 6.5% of NaCl. After incubation at 37 °C for five days, 2 mL of the culture were transferred to a new test tube containing 2 mL of 0.1 M HCl. The mixture was centrifuged at 15800 ×*g* for 5 min. Derivatization and HPLC method to analyze BA content was carried out based on Materials and Methods section 2.3.

Extraction of chromosomal DNA

The selected strains were inoculated in MRS broth and grown until their optical densities at 600 nm reached a value of approximately 1.0 at 37 °C. Aliquots of 2 mL of bacterial cultures were processed using TIA Namp Bacteria DNA kit DP302 (TIANGEN BIOTECH CO., LTD, Beijing, China). After washing with 70% ethanol, DNA was pelleted and resuspended in 50 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.6). The purified DNA extracts were separated by gel electrophoresis on 1% w/v regular G-10 agarose (BIOWEST, Spain) using DYCP-31BN electrophoresis apparatus (Liuyi,

Beijing, China). The running buffer was 1X TAE buffer (Tris acetate-EDTA, pH 8.0) buffer, and the voltage was set at 70 V. Results were visualized under a UV light using Ultra GelRed Nucleic Acid Stain (100-2000bp) (10000x) (Vazyme Biotech Co., Ltd, Nanjing, China).

Detection of tyrosine decarboxylase (tdc) gene

The presence of the tyrosine decarboxylase (*tdc*) gene was assayed by PCR reaction. The following primers were used: TD2 (5' ACTTAGTCAACCATRTTGAA 3') and TD5 (5' CAAATGGAAGAAGAAGTAGG 3') (18), obtaining an amplification product of 1100 bp. The PCR reaction was performed in 25 µL of reaction mixture containing 12.5 µL of 2×Taq PCR MasterMix KT201 (TIANGEN), 10 µM of primers (1 µL TD2, 1 µL TD5), 1 µL of DNA template and 9.5 µL of double-distilled H_2O. The amplification was performed using T100™ Thermal Cycler (Bio-Rad, UK) utilizing the following program: 4 min at 94 °C for initial denaturation, followed by 30 cycles at 94 °C for the 20s of denaturation, 55 °C for 30s of annealing, and 72 °C for 30 s of extension; a final extension step was carried out at 72 °C for 5 min. PCR products were separated by gel electrophoresis on 1% w/v regular G-10 agarose (BIOWEST, Spain) using DYCP-31BN electrophoresis apparatus (Liuyi, Beijing, China). The running buffer was 1X TAE buffer (Tris acetate-EDTA, pH 8.0), and the voltage was set at 70 V. PCR amplification results were visualized under a UV light using Ultra GelRed Nucleic Acid Stain (100-2000bp) (10000x) (Vazyme Biotech Co., Ltd, Nanjing, China).

16S rDNA bacterial identification

Bacterial strains containing *tdc* genes were identified using the PCR products obtained by universal 16S rDNA primers: 27F (5' AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' GGTTACCTTGTTACGACTT 3'). Amplicons were then purified and sequenced by MAJORBIO Co., Ltd (Shanghai, China). Sequence similarity was analyzed using BLAST search in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/).

Inoculation of BA-producing bacteria in sufu sample

10 g of sufu sample was homogenized using a high-speed blender (T25-basic, IKA, Germany) in 60 mL of DI water at 11,000 rpm for 3 min. The sufu suspension was sterilized at 121 °C for 15 min. A 100 µL aliquot of identified tyramine-producing strains was inoculated in 4.9 mL of the prepared sterilized sufu suspension. After incubation at 37 °C for 5 days, a 2 mL aliquot of the culture was transferred to a new test tube containing 2 mL of 0.1 M HCl. The mixture was centrifuged at 15800 ×*g* for 5 min. Derivatization and BA content analysis was carried out based on Materials and Methods section 2.3.

Statistical analysis

The significance of differences was determined by one-way analysis of variance (ANOVA) and Least-Significant Difference (LSD) (α= 0.05). Pearson's correlation coefficients between microbial, physicochemical indexes and BA concentrations were generated using Pearson's correlation coefficient analysis (α= 0.05). The statistical analyses were carried out using the IBM SPSS statistics package version 23.0 (SPSS Inc., Chicago, IL, USA).

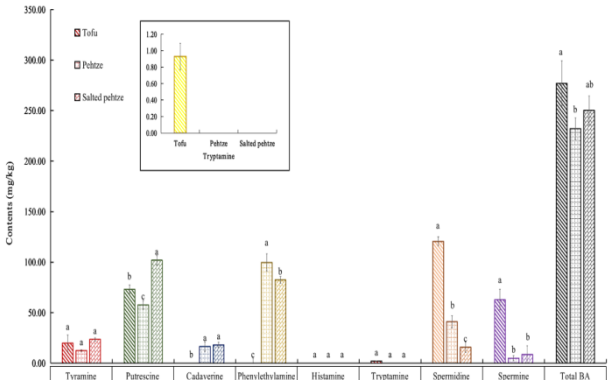
Results and discussion

Quantitative analysis of biogenic amines during sufu fermentation by HPLC

The calibration curves of standard biogenic amines showed good linearity (R^2> 0.9990) (Table S1). Figure 1 shows the contents of eight BAs in tofu, pehtze and salted pehtze during pre-fermentation. All these eight BA content analysis experiments were performed in triplicate. The most abundant BA detected in tofu were spermidine, putrescine, spermine and tyramine, the concentration of which were 120.54 ± 4.45 mg/kg, 73.13 ± 4.17 mg/kg, 62.77 ± 10.22 mg/kg and 19.69 ± 8.22 mg/kg, respectively. Previous results have indicated the existence of polyamines such as spermidine, spermine, putrescine. Arginine, one of the precursors of polyamine biosynthesis in soybean products, has been reported to be abundantly present in soybean. As most polyamines are readily water-soluble, they are likely found in tofu or tofu products (19). BA profile alters dramatically

throughout the fermentation process, as the concentration of slighter toxic endogenous BA decreases, while that of exogenous BA increase over time. Specifically, phenylethylamine and cadaverine levels increased significantly two days into tofu upon inoculation of a pure mold inoculum, while the contents of spermidine, putrescine, spermine decreased. Moreover, after salting for three days, the content of putrescine increased, and the content of spermidine decreased.

Spermidine was found to be the most prevalent BA in tofu but decreased in the latter fermentation process. This conclusion matches with the previous findings (20), confirming that the high spermidine content is related to that in the original soybean. In pehtze, the highest composition of BA is the phenylethylamine, which is proved to be a threaten to human health. In the salted pehtze, however, putrescine appears to be the most abundant BA, which is formed from the process of amino acid decarboxylation: arginine and glutamine combine to be the ornithine that decarbonates into the putrescine.



	Tyramine	Putrescine	Cadaverine	Phenylethylamine	Histamine	Tryptamine	Spermidine	Spermine	Total BA
Tofu	19.69±8.22a	73.13±4.17b	ND	ND	ND	ND	120.54±4.45a	62.77±10.22a	276.13±18.36a
Pehtze	12.40±0.69a	57.64±4.50c	16.51±4.73a	99.64±8.65a	ND	ND	41.09±6.18b	4.83±2.46b	232.10±10.83b
Salted pehtze	23.50±1.65a	101.82±4.71a	18.11±2.78a	82.38±3.22b	ND	ND	15.82±5.20c	8.60±8.53b	250.25±14.30ab

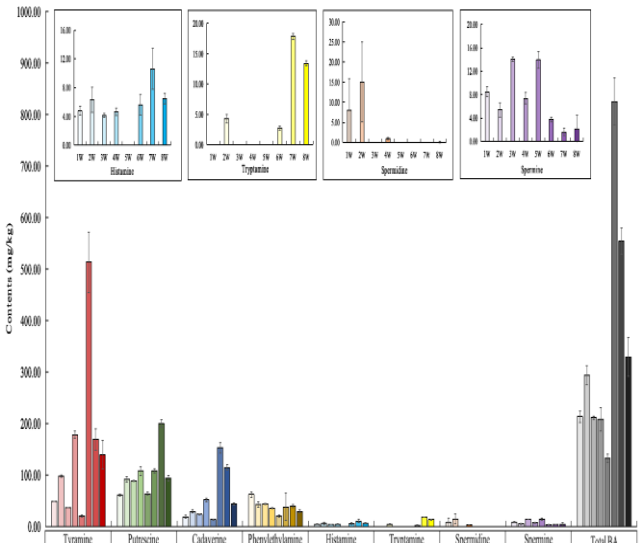
Figure 1. Changes of biogenic-amine concentrations in ▨ tofu; ▨pehtze; ▨salted pehtze during pre-fermentation. BA concentration throughout pre-fermentation was as follows: tyramine: red, putrescine: green, cadaverine: blue, phenylethylamine: bronze, tryptamine: yellow, spermidine: orange, spermine: purple and total BA: black. Expanded tryptamine content is shown in the inset. BA concentrations were expressed as mean value (mg/kg) ± standard deviation (mg/kg) in the table. Mean values for amines marked with the same superscript do not differ significantly ($p \geq 0.05$).

In Figure 2, BA levels rose to as high as 825.48 ± 46.15 mg/kg during ripening, indicating BA was mainly produced at the post-fermentation stage. During maturation, the total amount of BA fluctuates between 132.57 mg/kg to 825.48 mg/kg in sufu, the higher end being close to the elicit toxic level (1000 mg/kg) through oral consumption. Tyramine, putrescine, phenylethylamine and cadaverine were the main BA produced in white sufu during ripening. In general, BA levels increased the first four weeks during post-fermentation, decreased the fifth week before levels of tyramine, putrescine, cadaverine and total BA reached higher. Phenylethylamine concentration decreased from 62.41 ± 5.80 mg/kg in the first week to 20.29 ± 1.52 mg/kg in the fifth week and then rose to 39.77 ± 2.72 mg/kg in the seventh week. The final concentration of phenylethylamine at the end of post-fermentation was 29.64 ± 2.72 mg/kg, which was close to the oral intake threshold at 30 mg/kg. The putrescine level increased from 61.41 ± 1.37 mg/kg in the first week to 200.20 ± 7.23 mg/kg in the seventh week, before drastically dropping to 94.23 ± 5.25 mg/kg in the final week. Despite that the putrescine level fluctuated throughout the fermentation process, it remains above the oral intake threshold at 50 mg/kg. Additionally, the level of cadaverine increased from 19.05 ± 2.68 mg/kg in the first week to 51.86 ± 3.83 mg/kg in the fourth week and then fell from 153.26 ± 10.83 mg/kg to 44.08 ± 1.87 mg/kg at the end of the fermentation process. The content of tyramine increased sharply during ripening, indicating that tyramine was mainly produced in the post-fermentation stage. Specifically, the tyramine level reached 513.72 ± 58.98 mg/kg in the sixth week and decreased to 139.33 ± 27.40 mg/kg in the final week. Although the tyramine level was close to its oral intake threshold at 100 mg/kg, additional experiments would need to perform to assess the toxicity of sufu during storage due to the accumulation of tyramine.

Among all BA analyzed during sufu production, tyramine accounts for the largest portion of BA produced in sufu. Similarly, significant levels of tyramine and phenylethylamine have also been reported in Natto, a popular fermented soybean product in Japan (21). Moreover, most strains isolated from the fermented tofu including *Bacillus subtills*, *Bacillus tropicus*, and *Bacillus cereus* all produce tyramine (16). The high level of tyramine predominantly resulting from the *Bacillus subtilis* is highly capable of producing BA during fermentation.

(20) These findings indicate that tyramine is prevalent in fermented soybean foods and is worth investigating.

When it comes to other biogenic amines, putrescine, cadaverine and phenylethylamine appear to be the second, third and fourth-largest BA groups, respectively. According to the biological triplicate, the result is stable under this circumstance. However, this sequence of the BA content differs significantly in the previous studies when any slight changes in temperature, pH, salt concentration and additives take place. (22) Thus, the susceptibility sufu during ripening happened to be the limitation of traditional fermented soybean. Studying the biological changes in the fermentation process provides insights to standardize the product and helps the traditional sufu realize industrialization.



	Tyramine	Putrescine	Cadaverine	Phenylethylamine	Histamine	Tryptamine	Spermidine	Spermine	Total BA
1W	49.29±0.16	61.41±1.37	19.05±2.68	62.41±5.80	4.77±0.59	ND	8.01±7.93	8.49±0.90	213.43±10.84
2W	98.27±2.13	92.28±5.23	20.59±2.79	43.04±5.48	6.31±1.75	4.29±0.70	15.04±0.97	5.4±1.26	294.24±19.06
3W	37±0.15	88.6±1.01	23.67±0.23	44.04±0.99	4.14±0.26	ND	ND	14.06±0.36	211.51±2.89
4W	178.3±7.48	108.23±8.81	51.86±3.83	35.29±1.22	4.64±0.53	ND	0.94±0.00	7.37±0.96	386.63±22.83
5W	20.71±1.92	64±2.66	13.65±1.21	20.29±1.52	ND	ND	ND	13.92±1.42	132.57±8.03
6W	513.72±58.98	108.34±4.45	153.26±10.83	38.04±26.59	5.63±1.44	2.75±0.32	ND	3.74±0.40	825.48±46.15
7W	169.27±20.77	200.2±7.23	115.2±5.27	39.77±2.72	10.64±2.89	17.87±0.46	ND	1.61±0.60	554.55±26.31
8W	139.33±27.40	94.23±5.25	44.08±1.87	29.64±2.72	6.48±0.71	13.44±0.38	ND	2.13±2.36	329.47±37.05

Figure 2. Changes of biogenic-amine concentrations during sufu ripening. Weekly BA concentrations during ripening were as follows: tyramine: red, putrescine: green, cadaverine: navy, phenylethylamine: bronze, histamine: blue, tryptamine: yellow, spermidine: orange, spermine: purple and total BA: black. Color darkens as fermentation progresses. Expanded histamine, tryptamine, spermidine, spermine contents are shown in the inset. BA concentrations were expressed as mean value (mg/kg) ± standard deviation (mg/kg) in the table.

Relationship between physicochemical and microbial properties of biogenic amines contents during fermentation

pH, total acid and amino nitrogen content

Table 1. Changes in pH, total acid and amino nitrogen contents during sufu production.

Fermentation stages		pH	Total acid (g/100g)	Amino nitrogen(g/100g)
Pre-fermentation	Tofu	6.13±0.01^{c}	0.25±0.02^{a}	0.11±0.011^{a}
	Pehtze	5.82±0.02^{b}	0.61±0.02^{b}	0.22±0^{b}
	Salted Pehtze	5.37±0^{a}	1.22±0^{c}	0.38±0^{c}
Ripening	1W	5.94±0.01^{d}	0.5±0.03^{d}	0.13±0^{d}
	2W	6.1±0.01^{g}	0.5±0.03^{d}	0.23±0^{e}
	3W	6.37±0^{j}	0.43±0^{d}	0.27±0.03^{f}
	4W	6.14±0^{h}	0.67±0.03^{e}	0.38±0^{g}
	5W	6.32±0.01^{i}	0.6±0.05^{e}	0.35±0.01^{h}
	6W	5.94±0.01^{d}	0.64±0.06^{e}	0.4±0.02^{i}
	7W	6.08±0.01^{f}	0.75±0.08^{f}	0.52±0.02^{k}
	8W	6.06±0.02^{e}	0.64±0.05^{e}	0.48±0.02^{j}

Results expressed as mean±standard deviation; samples were measured in triplicates.

$^{a\text{-}c}$ in the row of pre-fermentation and $^{d\text{-}k}$ in ripening: Mean values marked with the different superscript differ significantly ($p<0.05$).

The changes in the chemical properties and compositions during different fermentation stages of sufu are reported in Table 1. At the pre-fermentation stages, pH decreases from 6.13 in tofu to 5.37 in salted pehtze. On the contrary, total acid and amino nitrogen contents rose from 0.25 g/100 g in tofu to 1.22 g/100 g in salted pehtze and from 0.11 g/100 g in tofu to 0.38 g/100 g in salted pehtze. Research on soybean fermentation has indicated that the presence of amino acids, mainly generated through primary proteolysis of starting material by endogenous proteases from microorganisms, which was subsequently hydrolyzed or converted into various derivatives, contributing to the decrease in pH and increase in total acid levels during fermentation (23). During the ripening period, the pH fluctuated slightly between 5.94 and 6.06. Salted pehtze contains the highest total acid level at 1.22 g/100 g, which decreased to around 0.50 g-0.67 g/100 g during the post-fermentation stage. The amino nitrogen content shows an increasing trend from 0.13 g/100 g in the first week to 0.52 g/100 g in the seventh week. During ripening, protease produced by various microorganisms introduced during pre-fermentation deliberately or accidentally broken protein down into

peptides and amino acids, further hydrolyzed to amines (23). The increase of total acid and amino nitrogen indicated the growth of organic acids and free amino acids during sufu production that provides precursors for BAs.

The total number of bacteria declined from 5.46 in the first week to 3.93 log CFU/g in the third week, possibly due to the presence of alcohol and salt in the dressing mixture that inhibited microbial growth. The microbial number climbed back to 5.23 log CFU/g in the final week, which could be because of the development of salt-tolerant microorganisms in the ripening stage.

Changes of total aerobic microbial and lactic acid bacteria content in sufu samples throughout ripening stages have been shown in Figure S1.

Table 2. Correlation between biogenic amines and microbial, chemical indexes [a]

		Total BA	TRP	PHE	PUT	CAD	HIS	TYR	SPD	SPM
pH	Pearson Correlation	0.160	-0.070	-0.045	0.203	0.066	0.178	0.150	0.094	-0.076
	Sig.(2-tailled)	0.488	0.762	0.847	0.376	0.778	0.441	0.516	0.685	0.744
Total acid	Pearson Correlation	0.168	0.032	-0.513*	0.119	0.284	-0.240	0.195	-0.714**	0.254
	Sig.(2-tailled)	0.479	0.893	0.021	0.617	0.225	0.309	0.411	0.000	0.280
Amino nitrogen	Pearson Correlation	0.524*	0.394	-0.609**	0.517*	0.613**	0.094	0.472*	-0.578**	-0.151
	Sig.(2-tailled)	0.018	0.086	0.004	0.020	0.004	0.693	0.036	0.008	0.525
Total aerobes	Pearson Correlation	-0.306	-0.240	0.198	-0.352	-0.048	-0.179	-0.152	-0.452	-0.162
	Sig.(2-tailled)	0.505	0.605	0.671	0.438	0.919	0.700	0.746	0.308	0.728
Lactic acid bacteria	Pearson Correlation	-0.427	-0.335	-0.112	-0.035	0.032	-0.367	-0.380	-0.705	0.323
	Sig.(2-tailled)	0.340	0.462	0.811	0.940	0.946	0.418	0.401	0.077	0.480

[a] TRP: tryptamine, PHE: β-phenylethylamine, PUT: putrescine, CAD: cadaverine, HIS: histamine, TYR: tyramine, SPD: spermidine, SPM: spermine. *Correlation is significant at the 0.05 level(2-tailed). **Correlation is significant at the 0.01 level(2-tailed)

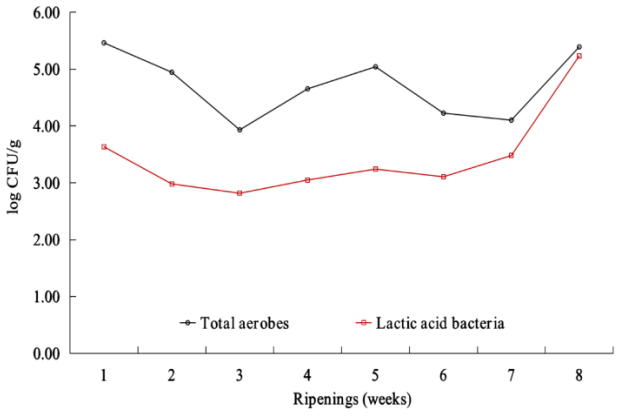


Figure S1. Changes of total aerobic microbial (black line) and lactic acid bacteria (red line) content in sufu samples throughout ripening stages.

Correlations between BA and microbial, chemical indexes

Correlation coefficients between microbial, physicochemical indexes and BA concentrations were generated using Pearson's correlation coefficient analysis (α= 0.05). Pearson's correlation test was conducted to examine correlations among the pH values, total acid, amino nitrogen, TMAB, LAB and BA contents of sufu during fermentation individually (Table 2). There is a positive correlation between amino nitrogen and the total BAs ($p<0.05$). Amino nitrogen refers to nitrogen in the form of amino acids, which is proportional to amino acids concentration. The result indicates that the production of BA is

closely related to the free amino acid level in sufu. The total acid content was significantly ($p<0.05$) related to phenylethylamine level and highly significantly ($p<0.01$) pertaining to spermidine level during sufu production with high Pearson's Correlation.

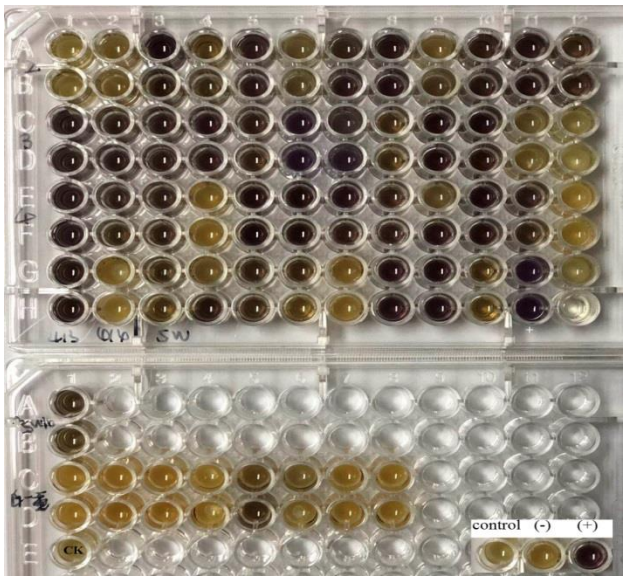


Figure S2. Screening for biogenic amine-producing bacteria using colorimetric method.

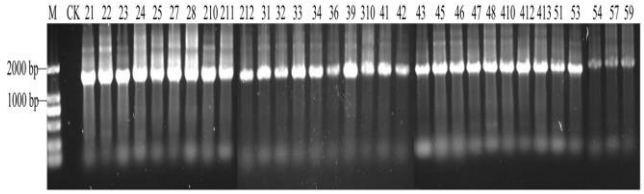


Figure S3. 16S rDNA electrophoresis of isolated strains.

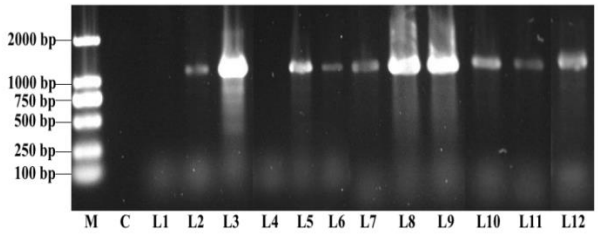


Figure S4. Electrophoretogram of representative PCR products (full-length gel is presented in Figure S2) of the TDC gene in selected strains. M:marker; C: control; L1-L3: PCR prduct from bacteria isolated from the 2nd week, L1: strain 21, L2: strain 22, L: strain 23; L4-L6: PCR product from bacteria isolated from the 3rd week, L4: strain 32, L5: strain33, L6: strain34; L7-L9: PCR product from bacteria isolated from the 4th week, L7: strain 43; L8: strain 45, L9: strain 46; L10-L12: PCR product from bacteria isolated from the 5th week, L10: strain 51, L11: strain 53, L12: strain 54.

Additionally, the amino nitrogen content was significantly ($p<0.05$) related to total BA content, putrescine and tyramine levels and is highly significantly ($p<0.01$) associated with phenylethylamine, cadaverine and spermidine with high Pearson's Correlations, the reason being that amino nitrogen compounds acted as precursors of the subsequent decarboxylation products BA. Results are consistent with a study showing that properties such as pH, salinity, and TMAB had little correlation with BA concentration during Cheonggukjang fermentation (24). As for microbial level, previous studies have shown that BA production depends both on the number of bacteria and the activity of bacterial decarboxylase, while the latter one has the dominant effect (25, 26).

According to previous research, the significance of differences was determined by one-way analysis of variance (ANOVA) and Least-Significant Difference (LSD) ($\alpha= 0.05$), thus differences with P values of <0.05 were considered statistically significant. (25) It was found that in Pecorino cheese samples, there was no linear relationship between the number of tyramine-producing bacteria and concentration of tyramine, indicating similar microbial distribution may lead to entirely different biogenic amines profiles in food products since environmental conditions, including the availability of precursors and microorganism's ability to produce BA also play important parts on the BA profiles in food products (26, 27).

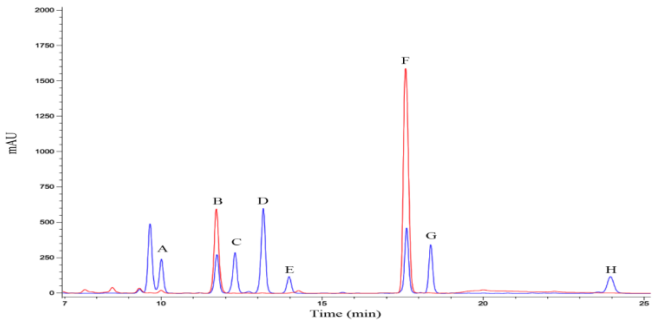


Figure S5. Representative HPLC chromatographic traces of BA standards (blue line) and BAs produced by isolated strain (Enterococcus faecalis 46) (red line). Chromatograph between 7 min to 25 min containing BA-of-interests was shown and the trace has been baseline corrected. A: tryptamine, B: phenylethylamine, C: putrescine, D: cadaverine, E: histamine, F: tyramine, G: spermidine, H: spermine.

Table 3. Biogenic amine produced by isolated bacteria in MRS broth.

Numbering	Biogenic amines content (μg/mL) b							
	TRP	PHE	PUT	CAD	HIS	TYR	SPD	SPM
WS-2-2	ND c	ND	2.16	ND	ND	8.24	ND	ND
WS-2-3	0.23	175.17	ND	0.15	1.10	695.27	ND	ND
WS-2-4	0.19	55.86	0.45	ND	ND	616.06	ND	ND
WS-2-5	ND	ND	ND	ND	ND	60.32	3.47	ND
WS-2-7	ND	89.45	ND	2.62	ND	772.96	5.56	ND
WS-2-8	0.23	77.02	ND	0.34	1.14	805.18	ND	ND
WS-2-10	ND	18.46	ND	ND	ND	563.51	12.3	ND
WS-2-11	0.08	77.61	0.25	ND	0.98	690.71	ND	ND
WS-2-12	0.78	106.63	ND	1.47	ND	728.12	6.44	ND
WS-3-1	0.66	144.33	ND	ND	ND	671.88	10.6	ND
WS-3-2	1.93	137.33	0.69	0.32	ND	645.12	11.7	ND
WS-3-3	ND	80.19	1.37	ND	ND	403.08	7.94	ND
WS-3-4	ND	135.72	0.43	ND	ND	670.46	ND	ND
WS-3-6	ND	11.23	0.27	3.89	1.19	821.128	ND	ND
WS-3-8	ND	92.39	ND	1.15	ND	783.407	1.32	ND
WS-3-10	ND	79.50	0.86	2.90	ND	724.82	ND	ND
WS-4-1	0.89	143.48	ND	0.63	0.25	683.57	ND	ND
WS-4-2	0.09	80.14	ND	2.04	ND	665.82	ND	ND
WS-4-3	0.10	78.14	1.11	2.37	ND	688.19	11.76	ND
WS-4-5	0.12	173.19	ND	3.10	ND	76.5	1.37	ND
WS-4-6	ND	148.47	1.25	1.44	ND	719.97	ND	ND
WS-4-7	ND	79.20	ND	1.39	ND	795.36	3.67	ND
WS-4-8	ND	104.06	ND	ND	ND	745.23	ND	ND
WS-4-10	ND	119.73	ND	0.29	ND	771.92	4.98	ND
WS-4-11	ND	83.15	ND	2.24	ND	726.73	2.85	ND
WS-4-13	0.18	133.50	ND	5.21	ND	773.38	2.73	ND
WS-5-1	ND	45.60	0.14	4.12	ND	758.26	ND	ND
WS-5-3	ND	49.17	ND	0.32	ND	663.76	5.45	ND
WS-5-4	ND	29.28	0.52	0.53	ND	620.76	5.61	ND
WS-5-6	0.25	79.71	ND	1.95	ND	677.21	3.11	ND
WS-5-7	ND	41.17	0.79	1.11	ND	713.77	3.25	ND
WS-5-9	ND	19.60	ND	1.33	ND	772.86	ND	ND

a Strain number description: for example, WS-2-2 represents the first strain isolated from the second week of post-fermentation; WS-3-3 represents the third strain isolated from the third week of post-fermentation.

b TRP: tryptamine; PHE: phenylethylamine; PUT: putrescine; CAD: cadaverine; HIS: histamine; TYR: tyramine; SPD: spermidine; SPM: spermine. c ND: not detected.

Table S1. Linear regressive equation and R^2 for calibration curve of biogenic amines.

Biogenic amines	Regressive equation	R^2
Tryptamine	y=27.093x+9.87677	0.9998
Phenylethylamine	y=33.097x+29.745	0.9999
Putrescine	y=90.714x+86.27	0.9999
Cadaverine	y=78.804x+68.99	0.9998
Histamine	y=61.054x+38.204	0.9998
Tyramine	y=55.103x-16.704	0.9998
Spermidine	y=75.677x+81.86	0.9997
Spermine	y=62.253x+39.814	0.9996

Identification of isolated tyramine and phenylethylamine-producing bacteria

Isolated strains from white sufu samples were subjected to three rounds of tests: colorimetric detection, HPLC and detection of tdc gene to identify biogenic amine-producing bacteria and eliminate false-positive results generated using any of the given methods. 54 strains of bacteria had been isolated in sufu, 32 of them were BA-producing strains (reaction showed purple color) based on the preliminary colorimetric screening (Figure S2). The ability and specificity for the isolated bacteria to produce BA in MRS broth was further characterized by HPLC (Table 3). Eight of the most prevalent BA were separated well on the HPLC chromatogram, indicating the chromatographic condition established in the study is sufficient in identifying and characterizing BA. *Enterococcus faecalis* WS-4-6, a strain isolated during post-fermentation as an example, was able to produce both phenylethylamine (173.19 μg/mL, Table 3) and tyramine (735.05 μg/mL, Table 3), with tyramine being the most predominant product. This may be a result of phenylethylamine, and tyramine being mainly produced by phenylalanine decarboxylase and tyrosine decarboxylase, respectively. Additionally, it has been shown that over-expression of a gene from *Enterococcus faecium*, which encodes a tyrosine decarboxylase in *Escherichia coli* results in both L-phenylalanine and L-tyrosine decarboxylase activity. Therefore, the production of aromatic biogenic amines could also be because *Enterococcus* amino acid

decarboxylase is able to catalyze the conversion to produce phenylethylamine and tyramine due to its broad substrate specificity.

As shown in Table 3, results have confirmed the ability of all isolated strains to produce tyramine in MRS broth. 30 out of the isolated strains (strains WS-2-3, WS-2-4, WS-2-7, WS-2-8, WS-2-10, WS-2-11, WS-2-12, WS-3-1, WS-3-2, WS-3-3, WS-3-4, WS-3-6, WS-3-8, WS-3-10, WS-4-1, WS-4-2, WS-4-3, WS-4-5, WS-4-6, WS-4-7, WS-4-8, WS-4-10, WS-4-11, WS-4-13, WS-5-1, WS-5-3, WS-5-4, WS-5-6, WS-5-7 and WS-5-9) had produced as high as 500 μg/mL tyramine in the media broth and exhibited excellent substrate-specificity. Additionally, out of the 30 strains that had the ability to synthesize phenylethylamine, 22 of them (strains WS-2-3, WS-2-4, WS-2-7, WS-2-8, WS-2-11, WS-2-12, WS-3-1, WS-3-2, WS-3-3, WS-3-4, WS-3-8, WS-3-10, WS-4-1, WS-4-2, WS-4-3, WS-4-5, WS-4-6, WS-4-7, WS-4-8, WS-4-10, WS-4-11 and WS-4-13. produced more than 50 μg/mL. Several isolated strains also had the ability to produce tryptamine (strains WS-2-3, WS-2-4, WS-2-8, WS-2-11, WS-2-12, WS-3-1, WS-3-2, WS-4-1, WS-4-2, WS-4-3, WS-4-5, WS-4-13 and WS-5-6), putrescine (strains WS-2-2, WS-2-4, WS-2-11, WS-3-2, WS-3-3, WS-3-4, WS-3-6, WS-3-10, WS-4-3, WS-4-6, WS-5-1 and WS-5-4), cadaverine (strains WS-2-3, WS-2-7, WS-2-8, WS-2-12, WS-3-2, WS-3-6, WS-3-8, WS-4-1, WS-4-2, WS-4-3, WS-4-5, WS-4-6, WS-4-7, WS-4-10, WS-4-11, WS-4-13, WS-5-1, WS-5-3, WS-5-4, WS-5-6, WS-5-7 and WS-5-9), as well as spermidine (strains WS-2-5, WS-2-7, WS-2-10, WS-212, WS-3-1, WS-3-2, WS-3-3, WS-3-8, WS-4-3, WS-4-5, WS-4-7, WS-4-10, WS-4-11, WS-4-13, WS-5-3, WS-5-4, WS-5-6 and WS-5-7). Overall, the tyramine level produced by isolated strains was higher than that of phenylethylamine. The highest tyramine level was 821.12 μg/mL, which was produced by strain WS-3-6. The lowest tyramine level produced by strain WS-2-2 was 8.24 μg/mL. As for phenylethylamine, the highest level was 175.17 μg/mL, produced by strain WS-2-3.

Since concentrations of two amino acid substrates (L-phenylalanine, L-tyrosine) were the same, there are two possible explanations as to why there are huge discrepancies between tyramine and phenylethylamine produced by the same microorganisms: (i) Tyrosine decarboxylase of these strains exhibits higher activity than their phenylalanine decarboxylase counterpart. (ii) tyramine decarboxylase hydrolyzes phenylalanine to phenylethylamine, to a lesser extent, due to possible enzymatic promiscuity.

Table S2. Identification and characterization of isolated bacteria based on BLAST results.

Numbering[a]	Strain	Identities (%)	Accession
23	*Enterococcus faecalis*	98	MG751341.1
25	*Pediococcus pentosaceus*	96	JN039348.1
27	*Pediococcus pentosaceus*	99	MF369881.1
211	*Enterococcus faecium*	97	JX317638.1
31	*Enterococcus faecium*	98	KX057668.1
33	*Enterococcus faecium*	96	MF369868.1
34	*Enterococcus faecium*	98	MG551256.1
36	*Enterococcus faecium*	99	KM497510.1
310	*Pediococcus acidilactici*	97	KJ779089.1
41	*Enterococcus faecium*	98	KJ026644.1
42	*Enterococcus faecium*	99	MG551256.1
43	*Enterococcus faecium*	98	KM497510.1
45	*Enterococcus faecalis*	99	MF369828.1
46	*Enterococcus faecalis*	97	MF369842.1
47	*Enterococcus faecalis*	99	KU922397.1
48	*Enterococcus faecalis*	99	KX057671.1
410	*Enterococcus faecalis*	98	MG543815.1
51	*Enterococcus faecium*	98	KF254539.1
53	*Enterococcus faecium*	98	JN560920.1
54	*Enterococcus faecium*	98	CP012430.1
56	*Enterococcus faecium*	98	KM495940.1
57	*Enterococcus faecium*	98	KM495940.1
59	*Enterococcus faecalis*	97	MF369862.1

a Strain number description: for example, 22 represents the first strain isolated from the second week of post-fermentation; 33 represents the third strain isolated from the third week of post-fermentation.

Table S3. Production of phenylethylamine and tyramine in sterilized sufu by isolated tyramine-producing bacteria (μg/mL).

Tyramine producing strains	β-Phenylethylamine	Tyramine
Enterococcus faecium 36 [Accession: KM497510.1]	62.49±5.57	71.05±1.44
Enterococcus faecalis 45 [Accession: KU922397.1]	76.67±1.53	71.19±4.64
Pediococcus acidilactici 310 [Accession: KJ779089.1]	70.99±9.55	118.64±32.71
Pediococcus pentosaceus 27 [Accession: MF369881.1]	27.03±6.73	75.94±6.03

Results are expressed as mean±standard deviation, samples were measured in triplicates; the results were corrected using the non-inoculated samples as controls.

In general, tyramine in food products is mainly produced through decarboxylation of tyrosine by bacterial tyrosine decarboxylase (TDC) (European Food Safety Authority (EFSA), 2011). Tyrosine decarboxylase requires pyridoxal-5-phosphate (PLP) for activity and thus belongs to the group of pyridoxal phosphate-dependent decarboxylase (18). In *Sporolactobacillus* sp. P3J, the TDC pathway is

encoded by a gene cluster consisting of four genes within which tdc, encodes tyrosine decarboxylase sits along with three other genes including tyrS, a gene homology to tyrosyl-tRNA synthetase gene, tyrP, encodes a tyrosine/tyramine exchanger, and nhaC, which is related to the Na+/H+ antiporter genes (28). Subsequently, the presence of tdc gene was also determined in these isolated strains. Results showed that bands of 23 of the isolated strains contain the same size as the target fragments, indicating they all harbor the tdc gene, as seen on the representative and full-length electrophoretograms in (Figures S3 and S4) (18). Identification of the 23 strains by 16S rDNA sequence analysis was then carried out to characterize isolated strains. As shown in Table S2, the sequences obtained were searched in the NCBI database; 20 strains of Enterococcus and three strains of *Pediococcus* species have been identified.

Representative HPLC chromatographic traces of BA standards and BAs produced by isolated strain have been shown in Figure S5.

Molecular identification and BAs production by identified tyramine-producing bacteria in sufu suspension

Based on Table S2, the following products were isolated and identified: 8 strains of *Enterococcus faecalis* (strains WS-2-3, WS-2-5, WS-4-5, WS-4-6, WS-4-7, WS-4-8, WS-4-10 and WS-5-9), 13 strains of *Enterococcus faecium* (strains WS-2-11, WS-3-1, WS-3-3, WS-3-4, WS-3-6, WS-4-1, WS-4-2, WS-4-3, WS-5-1, WS-5-3, WS-5-4, WS-5-6 and WS-5-7), one strain of *Pediococcus pentosaceus* (strain WS-2-7) and one strain of *Pediococcus acidilactici* (strain WS-3-10). To investigate biogenic amine production by *Enterococcus* and *Pediococcus* strains in sufu, *Pediococcus pentosaceus* WS-2-7 [Accession: MF369881.1] and *Pediococcus acidilactici* WS-3-10 [Accession: KJ779089.1], together with representative strains from *Enterococcus faecalis* and *Enterococcus faecium*, which are *Enterococcus faecium* WS-3-6 [Accession: KM497510.1] and *Enterococcus faecalis* WS-4-5 [Accession: KU922397.1] that showed the best abilities to produce tyramine and phenylethylamine, were chosen for the subsequent experiment in sufu suspension (Table S3). Table S3 showed that the tyramine produced by *Enterococcus faecium* WS-3-6 and *Enterococcus faecalis* WS-4-5 in sufu suspension were 71.05 μg/mL and 71.19 μg/mL, respectively which was similar to phenylethylamine produced by either strain. On the contrary, tyrosine conversion is much higher by *Pediococcus* species than phenylethylamine. *Pediococcus acidilactici* WS-3-10 produced 118.64 μg/mL tyramine, which is almost twice the level of phenylethylamine. Similarly, *Pediococcus pentosaceus* WS-2-7 produced 75.94 μg/mL tyramine, which is almost twice the level of phenylethylamine. The result confirmed isolated bacteria could produce BA in sterile sufu suspension.

Enterococcus occurred predominately during the ripening of sufu; it is one of the main constitutes of the sufu microflora (15, 16). *Enterococcus* is prevalent in the human and animal intestinal tract and can also be found in food, especially fermented food, like cheeses, fermented sausages and soy products (29). Studies have found that *Enterococcus faecium*, *Enterococcus faecalis* and *Enterococcus durans* isolated from red wine, fermented sorghum products were identified as tyramine-producing bacteria responsible for high tyramine contents in these food (30). Many studies show that tyramine biosynthesis is a species-level characteristic in *Enterococcus faecalis*, *Enterococcus faecium*, and *Enterococcus durans* since tyrosine decarboxylase present in *Enterococcus* genus bacteria could utilize both tyrosine and phenylalanine as the substrates to produce tyramine and phenylethylamine (30). Tyramine production is an essential way for *Enterococcus faecalis* to adapt to acidic environments. *Enterococcus* may play an important role in food fermentation due to its antimicrobial properties by production of a number of enterocins, which can inactivate spoilage and pathogenic bacteria (31). They may also have a positive effect on flavor-producing food by affecting citrate metabolism, lipolytic and production. However, the presence of *Enterococcus* in food may pose a risk as a result of BA production, causing antimicrobial resistances, and harbouring virulence factors (29).

Research regarding *Pediococcus pentosaceus* and *Pediococcus acidilactici*, as well as their abilities to produce tyramine, appear to be controversial. *Pediococcus pentosaceus* showed higher tyramine production in sauerkraut than *Lactobacillus Plantarum* or *Lactobacillus casei*. *Pediococcus acidilactici* has also been identified as a tyramine-

producing bacteria in cheese (32). At the same time, other groups show that *Pediococcus pentosaceu*s inhibits tyramine production in fermented sausages and wine (33).

Conclusion

In sum, to regulate the formation and accumulation of biogenic amines in fermented soy products, BA-producing bacteria were isolated and characterized in sufu; their decarboxylase activities were thoroughly investigated. Tyramine, putrescine, phenylethylamine and cadaverine were the main BA in sufu fermentation. Tyramine accumulation accounted for the most significant portion of BA produced in sufu, mainly made during ripening. Tyramine level increased drastically to reach 513.72 mg/kg during sufu ripening, posing potential health risks. During sufu fermentation, there was a positive correlation between amino nitrogen and BA, yet no significant correlation was found between BAs with pH and total acidity. In the present study, 23 strains of tyramine- and phenylethylamine-producing bacteria harbouring the tdc gene, including 20 strains of *Enterococcus* spp. and three strains of *Pediococcus* spp. were isolated and characterized in sufu. Among the isolated strains, *Enterococcus faecalis* WS-4-5, *Enterococcus faecium* WS-3-6, *Pediococcus acidilactici* WS-3-10 and *Pediococcus pentosaceus* WS-2-7 were characterized and exhibited excellent ability to produce tyramine in sufu suspension. To the best of our knowledge, the first report of tyramine-producing bacteria in sufu was presented. The current study provides insights into understanding the production of main BA in sufu by microorganisms.

Acknowledgments

None

Funding

This work was supported by Beijing Natural Science Foundation (Grant No 6204042), Beijing Key Laboratory for Food Non-Thermal Processing (NTKF2018004) and Xinghua Industrial Research Centre for Food Science and Human Health.

Conflicts interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contribution Statement

Yuwei Li and Tingting Yan performed the experiments and data analysis; Lijun Yin contributed to analysis and manuscript preparation; Yongqiang Cheng designed experiments and contributed to proofreading the manuscript; Xin Jia designed experiments, contributed to preparation and proofreading of the manuscript.

References

1. Farhang-Asa K, Eghbali Z, Khalili A, Derakhshan A, Karami A. Investigating the Production Potential of Soybean Cultivars Tolerant to Heat Stress from Brazil in Khuzestan Province. Agrotech Ind Crops 2021; 1(4): 188-196.
2. Ke RH, Wei ZS, Bogdal C, Goktas RK, Xiao RY. Profiling wines in China for the biogenic amines: A nationwide survey and pharmacokinetic fate modelling. Food Chem Jun 1 2018; 250: 268-275.
3. Linares DM, del Rio B, Redruello B et al. Comparative analysis of the in vitro cytotoxicity of the dietary biogenic amines tyramine and histamine. Food Chem Apr 15 2016; 197: 658-663.
4. del Rio B, Redruello B, Linares DM et al. The dietary biogenic amines tyramine and histamine show synergistic toxicity towards intestinal cells in culture. Food Chem Mar 1 2017; 218: 249-255.
5. Dolatparast B, Ahmadvand G, Mehrshad B, Hamzei J, Yazdandoost Hamedani M. Effect of Late Sowing Date on Agronomic and Quality Traits of Four Winter Oilseed Rape (Brassica napus) Cultivars in Hamedan, Iran. Agrotech Ind Crops 2021; 1(4): 160-169.
6. Sanlier N, Gokcen BB, Sezgin AC. Health benefits of fermented foods. Crit Rev Food Sci Feb 4 2019; 59(3): 506-527.
7. Noer ER, Dewi L, Kuo CH. Fermented soybean enhances post-meal response in appetite-regulating hormones among Indonesian girls with obesity. Obes Res Clin Pract Jul-Aug 2021; 15(4): 339-344.
8. Guan RF, Liu ZF, Zhang JJ et al.

Investigation of biogenic amines in sufu (furu): A Chinese traditional fermented soybean food product. Food Control Jun 2013; 31(2): 345-352.
9. Han BZ, Beumer RR, Rombouts FM, Nout MJR. Microbiological safety and quality of commercial sufu - a Chinese fermented soybean food. Food Control Dec 2001; 12(8): 541-547.
10. Kung HF, Lee YH, Chang SC, Wei CI, Tsai YH. Histamine contents and histamine-forming bacteria in sufu products in Taiwan. Food Control May 2007; 18(5): 381-386.
11. Qiu S, Wang Y, Cheng YQ, Liu Y, Yadav MP, Yin LJ. Reduction of biogenic amines in sufu by ethanol addition during ripening stage. Food Chem Jan 12 2018; 239: 1244-1252.
12. Tang T, Qian K, Shi TY et al. Monitoring the contents of biogenic amines in sufu by HPLC with SPE and pre-column derivatization. Food Control Aug 2011; 22(8): 1203-1208.
13. Yang J, Ding X, Qin Y, Zeng Y. Safety assessment of the biogenic amines in fermented soya beans and fermented bean curd. J Agric Food Chem Aug 6 2014; 62(31): 7947-7954.
14. Park YK, Lee JH, Mah JH. Occurrence and reduction of biogenic amines in traditional Asian fermented soybean foods: A review. Food Chem Apr 25 2019; 278: 1-9.
15. Huang XN, Yu SZ, Han BZ, Chen JY. Bacterial community succession and metabolite changes during sufu fermentation. Lwt-Food Sci Technol Nov 2018; 97: 537-545.
16. Liang JJ, Li DW, Shi RQ et al. Effects of microbial community succession on volatile profiles and biogenic amine during sufu fermentation. Lwt-Food Sci Technol Nov 2019; 114.
17. Poveda JM, Ruiz P, Sesena S, Palop ML. Occurrence of biogenic amine-forming lactic acid bacteria during a craft brewing process. Lwt-Food Sci Technol Nov 2017; 85: 129-136.
18. Coton M, Coton E, Lucas P, Lonvaud A. Identification of the gene encoding a putative tyrosine decarboxylase of Carnobacterium divergens 508. Development of molecular tools for the detection of tyramine-producing bacteria. Food Microbiol Apr 2004; 21(2): 125-130.
19. Righetti L, Tassoni A, Bagni N. Polyamines content in plant derived food: A comparison between soybean and Jerusalem artichoke. Food Chem Dec 15 2008; 111(4): 852-856.
20. Shi CS, Liu MM, Zhao HF, Liang LS, Zhang BL. Formation and Control of Biogenic Amines in Sufu-A Traditional Chinese Fermented Soybean Product: A Critical Review. Food Rev Int Jun 7 2021.
21. Kim B, Byun BY, Mah JH. Biogenic amine formation and bacterial contribution in Natto products. Food Chem Dec 1 2012; 135(3): 2005-2011.
22. Battistelli N, Tittarelli F, Perpetuini G, Suzzi G, Tofalo R. Occurrence of Biogenic Amines in Fermented and Non-fermented Soybean Products. Food Chem Funct Anal 2020; 20: 173-186.
23. Liu JJ, Han BZ, Deng SH, Sun SP, Chen JY. Changes in proteases and chemical compounds in the exterior and interior of sufu, a Chinese fermented soybean food, during manufacture. Lwt-Food Sci Technol Jan 2018; 87: 210-216.
24. Jeon AR, Lee JH, Mah JH. Biogenic amine formation and bacterial contribution in Cheonggukjang, a Korean traditional fermented soybean food. Lwt-Food Sci Technol Jun 2018; 92: 282-289.
25. Bai X, Byun BY, Mah JH. Formation and destruction of biogenic amines in Chunjang (a black soybean paste) and Jajang (a black soybean sauce). Food Chem Nov 15 2013; 141(2): 1026-1031.
26. Roseiro LC, Santos C, Goncalves H et al. Susceptibility of dry-cured tuna to oxidative deterioration and biogenic amines generation: I. Effect of NaCl content, antioxidant type and ageing. Food Chem Aug 1 2017; 228: 26-34.
27. Schirone M, Tofalo R, Fasoli G et al. High

content of biogenic amines in Pecorino cheeses. Food Microbiol May 2013; 34(1): 137-144.
28. Coton M, Fernandez M, Trip H et al. Characterization of the tyramine-producing pathway in Sporolactobacillus sp P3J. Microbiol-Sgm Jun 2011; 157: 1841-1849.
29. Chajecka-Wierzchowska W, Zadernowska A, Laniewska-Trokenheim L. Virulence factors of Enterococcus spp. presented in food. Lwt-Food Sci Technol Jan 2017; 75: 670-676.
30. Capozzi V, Ladero V, Beneduce L et al. Isolation and characterization of tyramine-producing Enterococcus faecium strains from red wine. Food Microbiol May 2011; 28(3): 434-439.
31. Yousif NM, Dawyndt P, Abriouel H et al. Molecular characterization, technological properties and safety aspects of enterococci from 'Hussuwa', an African fermented sorghum product. J Appl Microbiol 2005; 98(1): 216-228.
32. Bonetta S, Bonetta S, Carraro E, Coisson JD, Travaglia F, Arlorio M. Detection of biogenic amine producer bacteria in a typical Italian goat cheese. J Food Protect Jan 2008; 71(1): 205-209.
33. Garcia-Ruiz A, Gonzalez-Rompinelli EM, Bartolome B, Moreno-Arribas MV. Potential of wine-associated lactic acid bacteria to degrade biogenic amines. Int J Food Microbiol Aug 2 2011; 148(2): 115-120.