Occurrence of biogenic amines in sufu obtained from Chinese market

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Abstract This study aims at examining the level of biogenic amines (BAs) in different kinds of sufu commonly consumed in China. The correlation between different BAs and physical and chemical index in sufu samples was also investigated. The results proved that different processing technologies altered the distribution of BAs in commercial sufu. Total BA level was significantly correlated with salt content and pH. Some of the sufu samples in this survey contained higher levels of BAs, of which 26.6% of the samples might induce histamine poisoning, 15.6% might induce headache in virtue of phenylethylamine, and 23.4% might cause migraine and headache in virtue of tyramine. Moreover, 6.3% of the sufu samples with total BA content over 1000 mg/kg may be harmful to human health. From the food safety perspective, some sufu should not be excessively consumed daily and should be processed under strict sanitary conditions to decrease the BA level.

Keywords Biogenic amines · Sufu · HPLC · Physical and chemical indicators · Pearson's correlation coefficient

Introduction

Sufu (Fu-ru or Fermented bean curd) is unique and traditional fermented food in China; it has a history of 1500 years. In Europe and the United States, sufu is called “Oriental Cheese” because of its texture similar to soft cheese. Sufu has a unique flavor, delicious taste, delicate texture, rich nutrition and low price, which is very appreciated by consumers at home and abroad (Qiu et al., 2018). Sufu can be categorized into white, red, grey, paste, hot, and southern sufu according to its production method, color, or flavor, which can be used directly as a seasoning or as a seasoning for cooking food. Sufu is prepared from soybeans by the following method: assembly of tofu via salt precipitation from boiled soymilk; preparing of pehtze, which is a kind of tofu fermented by inoculation with pure culture; salting of pehtze; and ripening in dressing mixture (Qiu et al., 2018). Fermented sufu by microorganism has more nutrients and produces some physiological active substances that are useful to the human body, such as soybean peptide, soy isoflavones, and vitamin B₁₂ (Guan et al., 2013). The protein in sufu mainly exists in the form of soybean polypeptide, which can promote the decomposition of fat, reduce the content of cholesterol, and has the function of lowering blood pressure and antioxidant (Ahn et al., 2006). Soy isoflavones have anti-tumor effect, can effectively prevent and inhibit leukemia, and also have many biological activities, such as anti-oxidation and blood lipid lowering (Aresta et al., 2016). It is found that the total amount of isoflavones can reach the maximum during the late fermentation of sufu (Guan et al., 2013). The sufu produces a rich B vitamin in the fermentation process. Vitamin B₁₂ plays an important role in promoting human hematopoiesis, preventing pernicious anemia and senile dementia (Feng et al., 2003).

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Sufu contains a high content of protein which is suitable for the growth of microorganisms secreting amino acid dehydrogenase. It is easy to produce BAs during the long-term fermentation of sufu which leads to the potential insecurity of sufu (Guan et al., 2013). The predominant biogenic amine found in foods include ring putrescine, histamine, tyramine, cadaverine, tryptamine, phenethylamine, spermidine, and spermine. Most types of food contain low concentrations of BAs; low concentrations of BAs are beneficial to the human health. For example, putrescine and cadaverine have the effect of scavenging free radicals and lowering blood pressure; spermine and spermidine play an important role in regulating DNA, RNA and protein synthesis and biostability (Khairy et al., 2016). However, higher levels of BAs can cause some deleterious effects. Histamine has the greatest impact on human health, can cause diarrhea, vomiting, and may also cause respiratory disorders, palpitations, headaches (Chen et al., 2008). Tyramine can eliminate the nervous system, causing elevated blood pressure and migraine (Kalac and Krausova, 2005). In addition, the toxicity of between BAs also has additive and synergistic effects. For example, the presence of polyamines and diamines can inhibit the metabolic decomposition of monoamines, enhance toxicity, and easily cause poisoning events. Few BAs, such as cadaverine, putrescine, and other diamines, can react with nitrite to produce heterocyclic carcinogens, such as nitrosamines (Tassoni et al., 2000). Considering the possible harmful influences of BAs, monitoring the levels of BAs in sufu mainly consumed in China is necessary.

BAs are widely distributed in a variety of foods (Yang et al., 2014). There are a lot of studies on biosynthesis of BAs in food, but the research on BAs in fermented foods in China is still limited. Yen (1986) determined the content of BAs in fermented foods in Taiwan, tyramine (average content 485 mg/kg), putrescine (473 mg/kg), tryptamine (153 mg/kg), histamine (88 mg/kg), 2-phenylethylamine (63 mg/kg) and cadaverine (39 mg/kg) were detected in sufu. Kung et al. (2007) studied the BAs in fermented sufu and found that the BAs detected in 22 white and 10 red samples had putrescine, cadaverine, spermidine, spermine, tryptamine, histamine, tyramine and agmatine. The average content of histamine in white and red samples was less than 50 mg/kg, and the histamine content in only one red sample reached 158 mg/kg, but the content of BAs in different samples varies greatly. The content of BAs in fermented foods is related to many factors, and it is difficult to control BAs from one aspect. The involvement of microbes, the presence of amino acids, and the proper environment have a direct impact on the biosynthesis of BAs. Other factors affecting the biosynthesis of BAs in food were mainly concentrated in food materials, pH, temperature, NaCl concentration, selection of fermentation

strains, the fermentation time (Yatsunami and Echigo, 1993). In addition, it was found that the BAs of the samples with high amino acid nitrogen content were high, whereas the BAs content was low (Gardini et al., 2016).

The substrate of sufu is complex, and the method of determination of BAs needs to be further verified and improved. The investigation data of BAs in sufu is still limited. In the present study, we investigated BAs in 64 different brands of sufu samples obtained from Chinese markets by High Performance Liquid Chromatography (HPLC), and analyzed the edible safety of sufu. In addition, we analyzed the physical and chemical indexes which may affect the content of biogenic amine in sufu, and discussed its correlation with biogenic amine. This study provides a theoretical reference for the edible safety of many sufu products sold on the Chinese market.

Materials and methods

Sufu samples

A total of 64 sufu samples were purchased from local markets in Baoding City, Hebei Province, from 15 sufu making regions in China and were divided into six types. Per sufu sample with the same brand type and same production date were purchased three bottles. Based on the color, flavor, and dressing mixture, the sufu samples have been classified into the following six types: white sufu (19 samples), red sufu (18 samples), grey sufu (10 samples), paste sufu (5 samples), hot sufu (9 samples), and southern sufu (3 samples). The excipient mixture of these sufu samples consists of salt (sodium chloride), alcoholic beverage (yellow wine or white wine), angkak (red kojic rice), sesame oil, sugar, flour (soybean or rice) paste, and chili. All the sufu samples sold in the local supermarket were placed at room temperature.

Standards and reagents

The reagents used were of analytical grade, except for HPLC agential (acetonitrile) were acquired from Merck (Damstadt, Germany), which were of chromatographic grade. The water used was ultrapure and obtained by Millord-Q Plus system (Millipore Corp, Milford, MA, USA). The purity of standard BAs (tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine) and dansyl chloride (Dns-Cl) bought from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) was all above 98%.

Biogenic amine analysis

Preparation of standard biogenic amine solutions and sample extracts

The composition of the standard solution and the handling of the sample were slightly modified with reference to Lee et al. (2015). Eight biogenic amine standards were accurately weighted at 100 mg, dissolved with 0.1 M HCl, and then diluted to 25 mL to prepare a biogenic amine standard stock solution (4 mg/mL) for use. The same volume of the standard stock solution was pipetted, mixed, and diluted with 0.1 M HCl to obtain a final concentration of 500, 250, 100, 50, 25, 10, 5, and 1 µg/mL standard solutions. This solution was placed at 4 °C till its use for HPLC analysis.

The treatment of the sample was slightly modified with reference to Qiu et al. (2018). A total of 64 brands of sufu samples were lyophilized using a vacuum freeze tray dryer (Yanhua 610 L, Xinyang Quick Frozen Equipment Manufacturing Co., Ltd, China) and milled into powder. A portion of 2.0 g of ground sample was homogenized using a high-speed blender (Neofuge 15R; Lishen Co., Shanghai, China) at 19,000 r/min for 1–2 min with 20 mL of 0.1 M HCl, and the suspension was centrifuged at 7422×g for 30 min at 4 °C. The supernatant was filtered through a filter paper. 1 mL filtrate was absorbed into 5 mL centrifuge tube to be derived. Pipette 1 mL of filtrate into a 5 mL centrifuge tube for derivatization.

Sample derivatization

The derivative process was based on the methods of Pradenas et al. (2016). 1 mL standard biogenic amine solution or sufu sample extract solution was transformed into 5 mL centrifuge tube, and 200 µL of NaOH (2 M), 300 µL of saturated solution of NaHCO₃ and 1 mL of Dns-Cl solution (10 mg/mL in acetone) were added into the mixture. The mixture was placed in a water bath at 42 °C under dark conditions for 45 min. The unreacted Dns-Cl was then precipitated by the addition of 100 µL ammonia hydroxide at room temperature for 30 min in the dark. The volume of the reaction mixture was adjusted to 5 mL with acetonitrile and then centrifuged at 4 °C 3299×g for 10 min. The supernatant was filtered through a 0.45 µm organic phase needle filter (ANPEL LT., Shanghai, China Yuanye Reagent Co., Ltd) for HPLC analysis. All tests were performed in duplicate.

HPLC analysis

The quantification of BAs was conducted by Waters HPLC system (Watts Technology Shanghai, Co., China) comprising a model Waters 1525 pump and a model Waters

2489 ultraviolet–visible detector. Separation was performed by using an Agilent Eclipse XDB-C18 column (5 µm, 4.6 mm × 250 mm; Agilent Technology Co., US). 10 µL sample solution (filtrate) was injected into the solvent delivery system. The gradient elution program was set with a flow rate of 1.0 mL/min and the temperature was set at 40 °C. The elution was performed with 0.1 M ammonium acetate (solvent A) and acetonitrile (solvent B) using the following gradient elution program for separation: 0–7 min, 55–50% acetonitrile; 7–25 min, 50–90% acetonitrile; 25–35 min, 90–55% acetonitrile.

The suitability of the method for the determination of BAs in sufu samples was researched by means of linearity, sensitivity, accuracy, recovery and precision according to Tang et al. (2011). The calibration curves were prepared by determining the eight BAs concentrations from 1 to 500 µg/mL. Sensitivity was analyzed by detection and quantification limit. The spiked samples with different levels of the BAs were examined. A sufu sample with a known content of BAs were spiked at three levels (5, 12.5 and 25 µg/mL) of biogenic amine concentrations to evaluate their recovery. Three independent replicates were performed at each concentration for evaluating the accuracy. The precision of the determination method was assessed from three independent replicates for each biogenic amine. Intraday and interday repeatability of peak areas expressed as relative standard deviation (RSD (%)) were calculated.

Physical and chemical index analysis

Moisture content, A_w , and pH

The moisture content, A_w , and pH of the sufu samples were analyzed according to Han et al. (2001). The moisture content was measured by oven-drying method (100–105 °C for constant weight) of a 5.0 g sufu sample. A_w was directly determined with a water activity meter (Novasina CH-8533, AG Co., Switzerland). For the pH analysis, a 4.0 g sufu sample was homogenized at 16,000 r/min for 1 min with 20 mL deionized water, and then centrifuged at 4 °C 4750×g for 15 min. The supernatant was collected and diluted to 40.0 mL with deionized water. The sample pH was determined using a digital pH meter (Starter 3100; Ohaus Co., Shanghai, China).

Salt content

The salt content was determined by the molar method according to Qiu et al. (2018) and Wu et al. (2016). A 1.5 g sufu sample was boiled with 10 mL of distilled water with mild stirring. The sufu slurry was diluted to 50 mL with distilled water, and the supernatant was centrifuged at 4 °C

$7422 \times g$ for 15 min. The supernatant was then collected. A total of 1 mL of supernatant was mixed with 25 mL distilled water and titrated with 0.1 M AgNO_3 using 10% (w/v) K_2CrO_4 solution as indicator.

Amino nitrogen

Amino nitrogen was determined by formalin titration method with minor modifications (Guidi and Gloria, 2012). The sample solution (5 mL) was mixed with 30 mL water and titrated to pH 8.2 with 0.05 M NaOH before the addition 5 mL of 36% (w/v) formalin solution. Then, the mixture was titrated to pH 9.2 with 0.05 M NaOH. The volume of consumed NaOH for increasing pH (from 8.2 to 9.2) was taken to calculate amino nitrogen content.

Statistical analysis

Data were evaluated using descriptive statistics and the results were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was carried out by SPSS 22.0 (SPSS, version 22.0 for Windows, 2015; IBM Co, Somers, NY, USA). Data variance (ANOVA) and its significance were examined, and Duncan's multiple-range test ($p < 0.05$) was utilized to test the significance of differences in biogenic amine content and Physical and chemical index among the six categories of sufu samples. Pearson's coefficient ($p < 0.01$) was aimed at researching significant correlations between the contents of BAs and the physical and chemical index characteristics of the sufu samples. All tests were performed in triplicate.

Results and discussion

Evaluation of the method for biogenic amine determination

Linearity, sensitivity, repeatability, and recovery of the method were determined to evaluate the reliability and repeatability of the method. Figure 1 exhibits the chromatograms of BAs in standard solution and sample. The separation was evidently accomplished briefly (approximately 30 min), with a complete resolution for all the BAs peaks. The repeatability of the method was estimated by injecting a mixed concentration of biogenic amine standard solution (50 $\mu\text{g/mL}$) three times on the intraday (in the same day) and interday (during 7 days). The RSDs (Table 1) acquired were less than 1% and 7.5% for intraday and interday, respectively, to all BAs. The standard curve was determined from the peak area representation versus the levels of BAs. The linear range was determined by testing seven different levels of each of the BAs. Good

regression coefficients between 0.9972 and 0.9999 were obtained for all BAs (Table 1). The limit of detection (LD) was tested from the level of BAs ranged from 0.18 $\mu\text{g/g}$ for tryptamine to 0.65 $\mu\text{g/g}$ for spermine. The quantitative limits (LQ) of the BAs ranged from 0.60 $\mu\text{g/g}$ for tryptamine to 1.83 $\mu\text{g/g}$ for spermine.

The recovery of each biogenic amine was assessed by spiking a representative grey sufu samples; the levels of BAs were already determined with known contents of standard solution. Three determinations were made at each level of concentration. Standard BAs at concentrations comparable with those quantified were added, and the samples were subjected to the entire treatment procedure. As shown in Table 1, the recoveries of selected BAs were from 82.33 to 92.45% with RSD% from 0.61 to 3.40%.

All these determination results indicated that the extraction and derivatization procedures were reproducible and reliable. Thus, the HPLC method can be deemed a helpful tool for the quantification of BAs in sufu samples.

Investigation of the BAs in commercial sufu samples

The levels of BAs in six categories of 64 different commercial sufu samples are shown in Table 2. The content of BAs in 64 kinds of sufu samples was found to be 23.16–1151.39 mg/kg. The BAs of various types of sufu (white, red, grey, paste, hot, and southern sufu) were different. The content of BAs of the same type of sufu was different, and substantial differences exist between the levels of BAs. This finding may be due to the fermentation process of different degrees in the processing and production of sufu, the kinds of dressing mixtures, and processing conditions and environment (Han et al., 2001). Considerable differences were found in the levels of total BAs among white, red, grey, paste, and hot sufu. The total biogenic amine content in hot sufu was 162.71 ± 11.09 mg/kg, which was considerably lower than that in other types of sufu; the total biogenic amine content in white sufu and grey sufu was 533.54 ± 19.75 mg/kg and 758.21 ± 17.47 mg/kg, respectively, which was considerably higher than that in other kinds of sufu. The content of histamine, tryptamine, putrescine and cadaverine in grey sufu was significantly higher than that in other types of sufu ($p < 0.05$). These results might be due to the absence of alcoholic beverage in grey sufu, which has a certain inhibitory effect on the microorganism synthesis of BAs to some extent, and such results were in accordance with Qiu et al. (2018). The content of histamine (55.66 ± 2.67 mg/kg) in paste sufu and tyramine (19.62 ± 2.08 mg/kg), phenethylamine (8.09 ± 0.89 mg/kg) in hot sufu was considerably lower than that in other types of sufu, which may be related to the admixture of excipients and the processing conditions.

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

Table 1 Analytical characteristics with HPLC

BA	Linear regression	R ²	RSD (%) intraday	RSD (%) interday	LD (μg/g)	LQ (μg/g)	Spiked with standard BAs recovery (%)
TRY	$Y = 5550x + 23,300$	0.9998	0.13	4.56	0.18	0.61	92.45 ± 2.87
PHE	$Y = 6980x + 13,800$	0.9999	0.28	3.97	0.25	0.83	88.00 ± 1.62
PUT	$Y = 17,800x + 107,000$	0.9988	0.29	3.86	0.30	1.02	91.33 ± 0.61
CAD	$Y = 15,600x + 27,700$	0.9999	0.36	4.02	0.28	0.93	88.33 ± 2.06
HIS	$Y = 963x + 61,700$	0.9993	0.45	5.76	0.45	1.50	84.33 ± 3.40
TYR	$Y = 11,900x + 47,900$	0.9999	0.32	4.18	0.25	0.83	89.02 ± 2.58
SPD	$Y = 1050x + 109,000$	0.9996	0.53	6.23	0.30	1.00	86.33 ± 2.19
SPM	$Y = 29,900x + 230,000$	0.9972	0.39	7.49	0.65	1.83	82.33 ± 0.74

Values are expressed as mean ± deviation (n = 3); RSD relative standard deviation; LD detection limit; LQ quantitative limit

TRY tryptamine; PHE phenylethylamine; PUT putrescine; CAD cadaverine; HIS histamine; TYR tyramine; SPD spermidine; SPM spermine

Therefore, the effect of different processing technologies of sufu on BAs must be further investigated.

Occurrence (%) of BAs in sufu samples from the market was shown that tryptamine, phenylethylamine, putrescine, tyramine, and cadaverine were the most common BAs in 64 kinds of sufu samples, which was consistent with the results reported by Qiu et al. (2018). Tryptamine, phenylethylamine, tyramine, and histamine at the all sufu samples were detected in 93.75%, 98.44%, 98.44%, and 87.50%, respectively. Spermine was detected only in few

sufu samples. Histamine has the largest impact on human health, followed by the tyramine. According to Guan et al. (2013), cadaverine and putrescine were the common amines in white, grey, and red sufu samples, which can be related to poor hygienic conditions.

High contents of BAs in foods constitute a potential public health concern in virtue of toxicological effects. The contents of histamine in food were limited by South Africa (100 mg/kg), Australia (200 mg/kg), and European Community (100 mg/kg) (Bartowsky and Stockley, 2011;

Table 2 Contents of BAs in commercial sufu samples (mg/kg fresh weight)

BA	White sufu	Red sufu	Grey sufu	Paste sufu	Hot sufu	Southern sufu
<i>Tryptamine</i>						
Min	1.54	ND	13.05	12.12	ND	7.63
Max	212.52	74.78	139.02	76.26	18.88	51.35
Mean	48.21 ± 3.11 ^{ab}	14.29 ± 2.86 ^c	51.99 ± 2.06 ^a	45.33 ± 1.56 ^{abc}	5.23 ± 0.32 ^f	22.26 ± 1.51 ^d
<i>Phenylethylamine</i>						
Min	2.25	0.54	2.80	3.10	ND	10.60
Max	84.71	144.60	51.85	94.71	19.99	24.23
Mean	19.38 ± 2.26 ^{cd}	31.32 ± 1.05 ^b	22.12 ± 1.19 ^c	50.30 ± 3.37 ^a	8.09 ± 0.89 ^f	17.62 ± 2.89 ^{cde}
<i>Putrescine</i>						
Min	2.72	0.16	10.73	14.36	3.23	7.04
Max	264.68	110.33	220.42	80.23	92.28	65.09
Mean	88.12 ± 1.26 ^b	21.23 ± 2.85 ^c	106.35 ± 1.49 ^a	45.77 ± 2.64 ^c	27.18 ± 1.94 ^{de}	31.29 ± 1.71 ^d
<i>Cadaverine</i>						
Min	11.68	9.79	11.05	19.40	16.04	13.99
Max	606.47	46.01	104.06	32.51	54.39	28.77
Mean	31.92 ± 1.90 ^b	22.14 ± 2.65 ^{cde}	42.76 ± 2.91 ^a	22.72 ± 2.40 ^{cd}	28.08 ± 1.76 ^{bc}	21.96 ± 1.96 ^{cde}
<i>Histamine</i>						
Min	ND	ND	52.72	ND	ND	47.82
Max	537.07	728.40	616.28	175.88	251.29	312.63
Mean	205.12 ± 4.53 ^b	112.70 ± 4.69 ^d	322.61 ± 4.02 ^a	55.66 ± 2.67 ^e	66.66 ± 3.43 ^e	155.64 ± 2.21 ^c
<i>Tyramine</i>						
Min	0.54	1.97	21.93	105.94	ND	29.03
Max	370.93	242.39	319.24	276.53	113.74	164.93
Mean	113.04 ± 2.74 ^c	60.66 ± 2.04 ^e	141.37 ± 2.23 ^b	192.33 ± 4.55 ^a	19.62 ± 2.08 ^f	84.06 ± 2.88 ^d
<i>Spermidine</i>						
Min	ND	ND	ND	ND	ND	29.56
Max	89.96	71.01	117.18	6.13	25.60	108.47
Mean	27.75 ± 3.95 ^c	23.68 ± 2.00 ^{cd}	61.96 ± 3.48 ^{ab}	2.01 ± 0.14 ^e	7.84 ± 0.67 ^e	62.41 ± 2.12 ^a
<i>Spermine</i>						
Min	ND	ND	ND	ND	ND	ND
Max	–	1.47	48.32	–	–	–
Mean	–	0.08 ± 0.01 ^b	9.04 ± 0.09 ^a	–	–	–
Total amines	533.54 ± 19.75 ^b	286.10 ± 18.15 ^e	758.20 ± 17.47 ^a	414.12 ± 17.33 ^c	162.70 ± 11.09 ^f	395.24 ± 15.28 ^{cd}

Values are expressed as mean ± standard deviation (n = 3), ND not detected; BAs biogenic amines

Different letters (a–f) within the same line differ significantly, $p < 0.05$

Khairy et al., 2016; Latorre-Moratalla et al., 2017). The acceptable content of tyramine, histamine, and phenylethylamine in fermented foods were 0–100, 0–100, and 0–30 mg/kg, respectively (Moon et al., 2010). However, Santos suggested that the potentially toxic concentration of tyramine ranged between 100 mg/kg and 800 mg/kg, and the total BAs in food more than 1000 mg/kg is harmful to human health (Santos, 1996). Based on this criterion, the distribution of putrescine, phenylethylamine, tyramine, cadaverine, histamine, and total BAs in 64 kinds of sufu samples (fresh weight) were statistically

analyzed. The results are shown in Table 3, in which 26.6% of the sufu samples might account for histamine poisoning, 23.4% might induce migraine and headache due to tyramine, and 15.6% of samples might cause headache due to phenylethylamine. Moreover, 6.3% of sufu samples with total biogenic amine content over 1000 mg/kg may be harmful to human health. From the viewpoint of safety, some sufu should not be excessively consumed daily as a condiment; otherwise, it may be harmful to the human body.

Table 3 Distribution of major BAs in 64 kinds of sufu samples (fresh weight)

BAs levels (mg/kg)	Number of samples					
	PHE	PUT	CAD	HIS	TYR	Total BAs
< 30	54	37	41	18	28	2
30–100	9	13	22	29	21	8
100–800	1	14	1	17	15	42
800–1000	0	0	0	0	0	8
> 1000	0	0	0	0	0	4

PHE phenylethylamine; PUT putrescine; CAD cadaverine; HIS histamine; TYR tyramine

Analysis of correlation between different BAs and Physical and chemical index in sufu samples

The results of physical and chemical indicators of the six types of sufu are presented in this subsection. Table 4 shows that the water activity, moisture content, pH, salt content and amino nitrogen of all the samples were 0.83–0.89, 43.76–56.82%, 5.37–6.86, 1.80–3.77% and 0.32–0.76 g/100 g, respectively. The physical and chemical indicator of various types of sufu was different; the reason may be different from its raw material, fermentation temperature, fermentation strain and adjunct mixture. The pH (6.86 ± 0.02) and water content ($56.82\% \pm 0.64$) of grey sufu were considerably higher than those in five other types of sufu. The salt content of hot sufu was considerably different compared with that of other types of sufu. The amino nitrogen in grey sufu was substantially higher than those in four other types of sufu (except for paste sufu).

Pearson coefficient was used to test the correlation between biogenic amine content and physical and chemical indicator in sufu samples (Table 5). The results showed that there was a certain correlation between the physical and chemical index and the content of biogenic amine in sufu. Tryptamine was found to be significantly correlated with other BAs, except for spermidine, and

phenylethylamine was significantly correlated with tyramine. Furthermore, a notable positive correlation was determined between tyramine, histamine, cadaverine, and putrescine, and these results were similar to those acquired by other investigators (Herbert et al., 2005). Putrescine also demonstrated a marked positive correlation with spermidine and spermine due to the conversion of putrescine formation from arginine (Konakovsky et al., 2011).

The content of BAs was also related to physical and chemical indicators (Table 5). pH showed a positive correlation with tryptamine, putrescine, cadaverine, histamine, and spermine ($r = 0.692$ – 0.960). The effect of pH on BAs is also reported. Diaz-Cinco et al. (1992) studied the content of tyramine in cheese, and they found that tyramine synthesis was the fastest when pH was 5.0. Majjala et al. (1993) reported pH reduced rapidly by adding glucose- δ -lactone to raw ham meat. The results showed that the number of *Streptococcus faecalis*, aerobic thermophilic bacteria, and coliform bacteria were decreased. In addition, the contents of histamine and putrescine were also significantly decreased. Therefore, pH is a key factor to affect microbial growth and reduce the activity of amino acid degumming enzyme. In a certain range, adjusting substrate pH is an important way to control biosynthesis of BAs in food. Salt content also has an effect on the synthesis of BAs. In this study, salt content was significantly negatively correlated with tryptamine, phenylethylamine, putrescine, histamine, and tyramine ($r = -0.509$ to -0.869). The effect of salt content on BAs is also reported. Chin and Koehler (1986) studied the change of biogenic amine content in fermented paste with different salt content; it was found that 3.5–5.5% salt content could inhibit histamine synthesis. Yatsunami and Echigo (1993) found that 2% NaCl was added to sardines at 30 °C. The synthesis rate of histamine and cadaverine was very fast. Under the same conditions, 12% NaCl was added to sardines, and only traces amounts of histamine, putrescine and cadaverine were detected. The rate of synthesis of BAs is significantly inhibited. It can be seen that the high salt

Table 4 Determination of physicochemical property in sufu samples

Sample style	Total amines (mg/kg)	A_w	Moisture content (%)	pH	Salt content (%)	FAAN (g/100 g)
White sufu	533.54 ± 19.75^b	0.89 ± 0.01^a	52.63 ± 0.41^b	6.28 ± 0.02^b	1.96 ± 0.25^b	0.46 ± 0.05^b
Red sufu	286.10 ± 18.15^d	0.87 ± 0.01^a	53.14 ± 0.33^b	5.56 ± 0.02^d	2.05 ± 0.33^b	0.50 ± 0.04^b
Grey sufu	758.20 ± 17.47^a	0.89 ± 0.02^a	56.82 ± 0.64^a	6.86 ± 0.02^a	1.80 ± 0.24^b	0.70 ± 0.05^a
Paste sufu	414.12 ± 17.33^c	0.83 ± 0.02^b	43.76 ± 0.21^d	5.58 ± 0.01^d	2.01 ± 0.49^b	0.76 ± 0.06^a
Hot sufu	162.70 ± 11.09^e	0.87 ± 0.01^a	51.36 ± 0.30^c	5.93 ± 0.10^c	3.77 ± 0.29^a	0.41 ± 0.06^b
Southern sufu	395.24 ± 15.28^c	0.85 ± 0.01^{ab}	50.50 ± 0.13^c	5.37 ± 0.02^e	2.22 ± 0.21^b	0.32 ± 0.06^c

Values are expressed as mean $\pm$ standard deviation ($n = 3$)

^{a–f}Values with different superscripts represent significant differences within the same columns, $p < 0.05$

Table 5 Correlation between different BAs and physiochemical characteristics in the six types of sufu samples

Sample	Try	Phe	Put	Cad	His	Tyr	Spd	Spm	Aw	MC	pH	SC	FAAN
Try	1	0.503*	0.928**	0.729*	0.728*	0.891**	0.408	0.633*	0.318	0.198	0.692*	-0.722*	0.601*
Phe	0.503*	1	0.195	-0.035	-0.033	0.826**	-0.144	0.118	-0.528*	-0.545*	-0.094	-0.666*	0.817**
Put	0.928**	0.195	1	0.921**	0.889**	0.681*	0.531*	0.792*	0.609*	0.513*	0.894**	-0.541*	0.461
Cad	0.729*	-0.035	0.921**	1	0.931**	0.439	0.600*	0.927**	0.719*	0.714*	0.960**	-0.306	0.382
His	0.728*	-0.033	0.889**	0.931**	1	0.462	0.820**	0.880**	0.693*	0.773*	0.817**	-0.509*	0.222
Tyr	0.891**	0.826**	0.681*	0.439	0.462	1	0.26	0.476	-0.117	-0.167	0.358	-0.806**	0.763*
Spd	0.408	-0.144	0.531*	0.600*	0.820**	0.26	1	0.660*	0.384	0.646*	0.378	-0.449	-0.132
Spm	0.633*	0.118	0.792*	0.927**	0.880**	0.476	0.660*	1	0.49	0.622*	0.814**	-0.338	0.502*
Aw	0.318	-0.528*	0.609*	0.719*	0.693*	-0.117	0.384	0.49	1	0.904**	0.778*	-0.042	-0.155
MC	0.198	-0.545*	0.5138	0.714*	0.773*	-0.167	0.646*	0.622*	0.904**	1	0.664*	-0.084	-0.212
pH	0.692*	-0.094	0.894**	0.960**	0.817**	0.358	0.378	0.814**	0.778*	0.664*	1	-0.169	0.381
SC	-0.722*	-0.666*	-0.541*	-0.306	-0.509*	-0.806**	-0.449	-0.338	-0.042	-0.084	-0.169	1	-0.448
FAAN	0.601*	0.817**	0.461	0.382	0.222	0.763*	-0.132	0.502*	-0.155	-0.212	0.381	-0.448	1

MC moisture content; SC salt content; aw water activity; FAAN amino nitrogen content

* $p < 0.05$; ** $p < 0.01$ considered significant correlations

environment reduces the water activity of the sample, which leads to the deceleration of the growth and metabolism of the microorganism, thereby reducing the activity of the amino acid decarboxylase. Water activity and moisture content were significantly correlated with phenylethylamine, cadaverine, and histamine. The correlation between total biogenic amine content and physical and chemical index characteristics (date not shown) were also analyzed. The results showed substantial connection between salt content, pH value, and the total biogenic amine content, and the Pearson correlation coefficient was -0.706 and 0.721 . Therefore, the effects of salt content and pH on the production of BAs, which are key factors to minimize the biogenic amine level of sufu by reducing the activity of amino acid decarboxylase, may require further study in sufu production.

Reducing the content of BAs and some associated health risks in sufu will contribute to worldwide acceptance of sufu. According to the conditions of biogenic amine formation, the content of biogenic amine can be controlled by the following ways: controlling the content of free amino acid in food, controlling the growth of microorganisms producing amino acid decarboxylase, controlling the activity of amino acid decarboxylase, and enhancing the degradation level of biogenic amine. Therefore, it is necessary to further study the effects of different fermentation, processing conditions, storage conditions and storage time on the BAs biosynthesis in traditional fermented sufu products in China, so as to provide a theoretical basis for ensuring the safety of traditional fermented sufu products in China.

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