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Research Article

Risk Assessment of Biogenic Amines of Chinses Commercial Soy Sauce on Human Being via High-Liquid Performance Chromatography

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Abstract

Biogenic amines are basic nitrogenous compounds formed by decarboxylation of amino acids. Commonly they are present in all types of food and fermented soy sauce is a one common source of them. They have important role in hormone and protein synthesis but food containing high concentration of biogenic amines may have adverse effects. In the current study we investigated the toxicological effects of biogenic amines on human health found in chinses soy sauce by high-performance liquid chromatography. The levels of biogenic amines were investigated by using 50 samples of commercially available chinses soy sauce by HPLC with the spectrometric UV-via 254 nm using gradient elution. In this study, eight biogenic amines were detected. The concentration of biogenic amines was 3.67 g/L range from the 0.08 g/L to 16.10 g/L. The pH of the soy sauce samples was 4.28 and 5.43, and the salt levels in these samples were 10.21 g/100 mL and 21.68 g/100 mL.The contents of amino nitrogen and formaldehyde-reactive nitrogen-nitrogen were 1.00-2.55 g/L and 5.71-18.44 g/L, respectively. The amino nitrogen was observed high in samples. Therefore, it is significant to analyze the biogenic amine in soy sauce to assure their safety and prevention for human being consumption.

Keywords: Biogenic amines; Soy sauce; HPLC; Human; Risk assessment

Introduction

Due to nitrogenous and low molecular-weight and organic base, biogenic amines (BAs) are usually formed through the microbial decarboxylation of amino acid, especially in fermented soybean food such as sauce, cheese, kimchi, wine, miso, fermented seafood and meat products. BAs are endogenous and all essential nitrogenous components of living cells, and the

most common BAs found in food are tryptamine (TRP), tyramine (TYR), spermidine (SPD), spermine (SPM), 2-phenylethylamine (PHE),cadaverine (CAD), histamine(HIS), putrescine (PUT). BAs are generated by the effects of enzymes in raw material or amino acid decarboxylase that produced by microorganism metabolism. BAs are manufactured in the fermented food industrial product mainly affected food constitution, microbial flora, environmental factors and other parameters that has been allowed microbial germination during the food processing and handling [1,2].

The presence of BAs in the food compositions potential public health related due to toxicological and physiological defects. Low concentration of BAs is essential for many body functions, e.g., histamine plays important roles as neuromodulators present at generally low levels in mammals and the aliphatic polyamine. spermidine(SPD), putrescine,(PUT) and spermine (SPM) are usually cells components that play key roles in cell propagation and differentiation [3,4]. However, genesis of BAs in food by the decarbooxylation of amino acid carried out by bacteria as a result accuring of different allergen reaction and diseases in consumers likes diffculty in breathing, itching, fever, vomoting, increase the blood pressure and hypertension can be lead to brain hemorrhage and heart failure [5,6]. Moreover, BAs posing health risk as toxicity, cerebral hemorrhage and death in case of their ulitizaion in large quanitity at daily food [7] or naturally process the metabolism of amine is reserved or herediatry difecint. Furthermore, some BAs can react with nitrite which are become carcinogenic nitrosamines. Soy sauce extensively used in south asian countries like japan, vetanam, combodia, thiland, china as well in the westen countries [8]. Its popularity in all over the world is growing due to it's distinct falvor and aroma [9].The yearly production of soy sauce are 5 million metric tons in china, more than 50% of the world manfactured and production [10], and the demand for soy sauce utilzation is incresed round about 5 to 10% yearly [11]. In china soy sauce is manufactured by using soybean (defatted soybean) and wheat (bran) as a raw material and brewing through the process of microbial fermentation, enzymatic action, and non-enzymatic action. It is a kind of essential flavor in China and Asian diet. Thus its purity is closely related to people's health. The fermentation process of soy sauce mainly occurs in the process of koji-making, a method of combined activities of microbial flora, in which the generation of protease, hydrolyze protein and release of amino acid occured . Due to the fermentation effects from a variety of microorganism, soy sauce can contain relatively high content of BAs in a long fermentation process, which may lead to the potential insecurity of soy sauce [7, 12]. However, there are only a few reports about the BAs content is in soy sauce sold in the Chinese market [13]. For understanding the taxological and physiological effect of BAs of chinses soy sauce on human health a deep investigation was done in the present study and different BAs were identified to understand the correlation between BAs concentrations, the levels of amino nitrogen content, the levels of formaldehyde-reactive nitrogen content and the levels of pH and salt concentrations of chinses commercial soy sauce.

Methods and Materials

Samples

A total no of 50 samples of Chinese traditional soy sauce were analyzed (enlisted in Table 1). These 50 samples were purchased from different 6 regions of china including Harbin, Jinan, Tianjin, Beijing, Shanghai, Guangzhou and Zhengzhou, and of different 16 commercial brands. Total 40 samples were fermented with high salt and 10 were fermented with low salt solid conditions. All samples were transported to the laboratory under hygienic condition and preserved at -20°C.

Regents

The regents were analytical grades which are used in the experiments, except HPLC (acetonitrile) which were grade by chromatographic. Tryptamine hydrocholride (TRP), tyramine hydrocholride (TYR), spermidine tri-hydrochloride (SPD). spermine tetra-hydrochloride (SPM), 2-phenyl ethylamine (PHE), cadaverine dihydrocholride (CAD), histamine dihydrocholride (HIS), putrescine dihydrocholride (PUT), and Dansyl chloride were obtain from the sigma Chemical Co. (St. Louis, MO, USA).

Table 1: List of the analyzed soy sauces samples and the levels of biogenic amine contents (g/L), salt contents (g/100 mL), amino nitrogen contents (g/L), formaldehyde-reactive nitrogen (g/L), pH and brewing process in tested soy sauces.

Samples [a]	Tryptamine[b]	2-Phenylethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine	Total content nitrogen contents	pH	Salt contents	Amino nitrogen contents	Formaldehyde-reactive nitrogen contents	Brewing process
Harbin														
01HXJY	nd	0.17±0.02	0.07±0.00	nd	0.44±0.04	0.72±0.06	0.19±0.00	nd	2.44		4.44	16.02	1.42	HLF
02TQHJY	nd	0.15±0.01	0.06±0.01	nd	0.41±0.05	0.62±0.10	0.19±0.12	nd	2.31		4.28	17.4	1.41	HLF
03ZXJZ	nd	0.62±0.06	0.03±0.00	nd	0.19±0.01	1.41±0.14	0.25±0.04	nd	3.9		4.38	15.97	1.98	HLF
04JXHD	nd	0.05±0.00	0.14±0.02	nd	0.32±0.02	0.80±0.05	0.1±0.010	nd	2.16		4.45	14.69	1.01	HLF
05JY	0.13±0.00	0.20±0.04	0.19±0.04	0.18±0.02	0.56±0.05	1.35±0.49	0.31±0.02	nd	4.72		4.54	13.93	1.48	HLF
06TJJY	nd	0.85±0.02	0.10±0.01	nd	1.05±0.01	1.26±0.01	0.25±0.02	nd	5.29		4.49	14.03	1.79	HLF
07TXJY	nd	0.35±0.01	0.53±0.00	0.62±0.01	1.00±0.01	1.13±0.13	0.18±0.09	nd	5.85		4.36	17.6	1.24	LSF
01YZX	nd	0.17±0.01	0.12±0.00	0.20±0.00	0.31±0.00	0.56±0.02	0.18±0.02	nd	2.33		5.25	12.71	1.86	HLF
02ZXJY	nd	0.71±0.05	0.10±0.05	nd	0.26±0.02	1.77±0.07	0.14±0.00	nd	4.57		4.83	13.16	1.88	HLF
Jinan														
01WJX	nd	0.09±0.03	0.08±0.01	0.16±0.02	0.25±0.04	0.39±0.02	0.14±0.09	nd	1.77		5.02	15.66	1.4	HLF
02YPX	nd	0.24±0.03	0.07±0.01	nd	0.33±0.02	0.38±0.05	0.05±0.01	nd	1.65		4.66	16.22	1.36	HLF
03HXJY	nd	0.34±0.02	0.06±0.00	nd	0.55±0.04	0.58±0.12	0.13±0.01	nd	2.59		4.66	12.65	1.39	HLF
04LC	nd	0.09±0.00	0.05±0.01	nd	0.56±0.02	0.87±0.09	0.25±0.04	nd	2.8		4.66	14.49	1	HLF
01TJ	nd	nd	0.07±0.01	0.08±0.01	0.05±0.00	0.13±0.01	0.17±0.01	nd	0.76		5.14	12.73	1.56	HLF
01WJX	nd	nd	0.02±0.00	nd	nd	nd	0.09±0.01	nd	0.18		5.06	12.45	2.35	HLF
01JX	nd	0.09±0.01	nd	nd	nd	nd	0.12±0.02	nd	0.34		4.94	12.3	2.33	HLF
Tianjin														
01JY	nd	nd	0.09±0.02	nd	0.11±0.02	0.08±0.03	0.12±0.03	nd	0.65		4.39	14.95	1.29	LSF
Beijing														
01LBJY	nd	nd	0.07±0.01	0.04±0.00	0.10±0.02	0.19±0.01	0.13±0.00	nd	0.82		4.59	14.95	1.37	HLF
Guangzhou														
01HDJY	nd	0.18±0.06	0.06±0.02	nd	0.35±0.10	0.38±0.00	0.04±0.00	nd	1.6		4.43	13.47	2.11	LSF
02HDLC	nd	0.42±0.04	0.62±0.03	0.13±0.02	1.43±0.06	1.38±0.17	0.09±0.00	nd	6.26		4.43	12.09	1.55	LSF
03HDSC	nd	0.71±0.06	0.09±0.00	0.10±0.00	1.03±0.01	1.03±0.26	0.1±0.01	nd	4.78		4.34	16.22	1	LSF
01HDJY	nd	0.51±0.01	0.91±0.04	0.15±0.01	1.59±0.06	2.11±0.13	0.1±0.02	nd	8.17		4.5	13.16	1.39	LSF
01YH(LW)	nd	0.27±0.02	0.21±0.02	0.14±0.02	0.60±0.08	1.08±0.11	0.37±0.01	nd	4.14		4.62	10.82	1.92	LSF

02YH(TX)	nd	0.32±0.04	0.06±0.01	0.11±0.03	0.23±0.01	0.79±0.13	0.05±0.00	nd	2.44	4.71	10.21	1.96	LSF
Shanghai													
01HDJY	nd	nd	0.03±0.00	0.16±0.02	nd	nd	0.04±0.01	nd	0.35	4.42	16.99	1.51	HLF
04HS	nd	0.77±0.02	0.78±0.00	0.27±0.00	1.14±0.02	1.31±0.07	0.05±0.00	nd	6.55	4.82	14.13	1.64	HLF
05LB	nd	0.04±0.00	0.29±0.02	0.09±0.00	0.33±0.04	0.53±0.04	0.15±0.09	nd	2.24	5.02	13.67	1.91	HLF
06HX	nd	1.80±0.00	0.67±0.01	0.26±0.00	1.60±0.03	2.13±0.41	0.09±0.02	nd	10.07	4.89	16.17	1.57	HLF
08YPX	nd	0.52±0.06	0.04±0.00	nd	1.15±0.11	1.01±0.27	0.09±0.01	nd	4.44	5.15	14.34	1.58	HLF
09WJX	nd	nd	0.44±0.00	nd	0.41±0.01	0.44±0.05	nd	nd	1.96	5.11	14.44	1.32	HLF
10CGLC	nd	0.14±0.03	0.28±0.04	nd	0.47±0.05	0.70±0.15	0.06±0.01	nd	2.61	4.89	20.46	1.07	HLF
11WJSC	nd	0.09±0.00	0.07±0.00	nd	0.39±0.01	0.39±0.08	0.08±0.01	nd	1.59	4.66	15.77	1.37	HLF
01LCW	nd	nd	0.05±0.01	nd	nd	0.06±0.01	nd	nd	0.18	4.84	21.68	1.85	HLF
03HD	nd	0.69±0.02	0.62±0.02	0.25±0.01	1.61±0.04	1.95±0.21	0.1±0.00	nd	7.97	4.63	18.06	2	HLF
04HD	nd	0.66±0.04	0.08±0.02	0.15±0.01	0.60±0.07	1.84±0.13	0.14±0.01	nd	5.34	4.63	15.77	1.32	HLF
05BWX	nd	0.38±0.04	0.03±0.00	nd	0.07±0.00	0.24±0.00	0.07±0.00	nd	1.22	4.75	15.46	1.2	HLF
06YPX	nd	0.42±0.02	0.03±0.01	nd	0.13±0.00	0.55±0.05	0.16±0.09	nd	2.03	5.06	12.45	1.6	HLF
07HX	nd	0.22±0.00	0.06±0.01	0.23±0.03	0.19±0.02	0.46±0.18	0.15±0.00	nd	2.08	4.8	15	1.39	HLF
08LB	nd	nd	nd	nd	nd	nd	0.1±0.02	nd	0.15	4.58	12.6	1.13	HLF
09HS	nd	nd	0.03±0.00	nd	nd	nd	0.14±0.01	nd	0.25	4.82	16.84	1.51	HLF
02LC	nd	nd	nd	nd	nd	nd	0.06±0.00	nd	0.08	4.83	17.5	1.47	HLF
03TCYW	nd	0.14±0.00	0.05±0.00	nd	nd	0.22±0.01	0.21±0.11	nd	1	4.97	15.77	1.41	HLF
04JXSC	nd	0.59±0.07	0.36±0.04	0.64±0.04	nd	1.26±0.11	0.22±0.01	nd	5.21	4.88	15.66	1.61	HLF
05JZSC	nd	0.10±0.03	0.06±0.03	nd	nd	0.20±0.07	0.22±0.00	nd	0.95	4.88	17.5	1.69	HLF
01LCW	nd	0.16±0.00	0.30±0.02	0.15±0.02	nd	1.38±0.22	0.17±0.00	nd	5.41	5.43	18.72	1.81	HLF
02HX	nd	1.05±0.13	0.12±0.04	0.14±0.01	nd	2.08±0.27	0.42±0.01	nd	6.82	4.77	15.92	1.15	HLF
03JLHX	0.17±0.00	0.87±0.78	1.10±0.86	0.23±0.00	nd	2.15±0.68	0.12±0.03	nd	9.21	4.81	17.3	2.49	HLF
01LCW	0.14±0.01	0.28±0.02	0.18±0.01	nd	nd	6.22±0.01	0.17±0.00	nd	16.1	5.07	17.4	2.53	HLF
01WJX	0.14±0.02	1.03±0.80	1.17±1.01	0.25±0.00	nd	4.68±1.10	0.13±0.00	nd	16.01	5.15	13.78	2.55	HLF
Zhengzhou													
01MTX	nd	0.14±0.00	0.27±0.01	nd	nd	0.15±0.01	0.21±0.02	nd	1.29	4.94	16.89	2.32	HLF

[a]Letters in the latter signify brands of soy sauce of each region. Numbers in the former signify different samples.

[b]Values represented the average of duplicates (n=2) ± standard deviation.

[c]Total contents of the eight BAs including tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine.

[d]HLF, high salt liquid state fermentation and LSF, low salt solid state fermentation.

[e]nd, not detected or below detection limits; mean values were calculated by using zero for nd.

Preparation of standard amine solution

Out of these stock solutions 8 standard biogenic amines (TRP), (TYR), (SPD), (SPM), (PHE), (CAD), (HIS) and (PUT) were individually prepared at 1mg/1ml concentration in 0.1 mol/L HCl. Working solution were prepared by the dilution of 1000 µl of each standard amine stock solution 0.1 mol/L HCl to obtain the final volume of solution 10 ml. These solutions were kept and store at 4° C until for further uses.

Biogenic amines determination

The content of the biogenic amines of soy sauce were invested according to methods and procedure formerly reported by Lu et al. Briefly, 90 ml of 0.6 M perchloric acid was mixed to 10 ml of soy sauce samples. One ml of standard amine solution was added with 2 M of sodium hydrochlorides and sodium hydrogen carbonate to adjust the pH of the solution up to 10.5. 2 ml of Dansyl chloride solution (10 mg/ml in acetone) was mixed in the mixture and incubated in the water bath at 40° C for 45 minutes. 100 µ of 25% ammonium hydroxide was added to stop the chemical reaction and eliminate residual dansyl chloride. After 30 min incubation at room temperature, the final volume was balance to 5 ml by mixing acetonitrile. Ultimately, the mixture was centrifuge at 3000 rmp for 5 minutes and the supernatant was filtered through 0.2 µm orifices size filter. The filter supernatant was stored at -25° C untill trail by HPLC.

Quantitative analysis of biogenic amines was carried out by using HPLC unit, Consisted of 2 pumps and a UV-vis detector device. Separation was got using a C18 column 5 µm (250 mm×4.6 mm). The mobile phase was ammonium acetate (0.1 M solvent) and acetonitrile (Solvent B) at the flow rate of 1 ml/min with gradient elution, program for 35 min mention in (Table 2). The samples volume was injected 10 µm. The samples were observed at 245 nm. Each HPLC run and look after about 35 minutes and after that the column was conditioned again with the maximum of 65% solvent A and35 % solvent B.

Table 2: Gradient elution program.(flow rate 1. 0mL/min)

Time (min)	A (%)	B (%)
0	65	35
5	55	45
10	35	65
16	20	80
25	10	90
35	65	35

(A) Ammonium acetate; B acetonitrile.

Determinations of amino nitrogen, pH, Salt and Formaldehyde reactive nitrogen

Calculation of pH, salt, formaldehyde reactive nitrogen and amino nitrogen: to determine and measure the different 25 samples deionized one, the distilled water was added to the 5 gm sample of soy sauce and the sample was homogenized and filtered with the help of Whatman paper. According to the method the pH was determined and was developed by using previously pH meter (Satorius PB_10) [14].

With the help of previous described method of (AOAC 1999) salt content was determined. The 20 ml sample was diluted with the addition of 180 ml distilled water. 10 ml of 0.1 N$AgNO_3$ and 10 ml of concentrated $HNO_3$was mixed to the diluted sample. The sample mixture was heated with low temperature on hot plate up to dissolved (normally for 10 minutes) apart from $Agcl_2$. Then after that in the sample cool running water was added for cooling. The 50 ml distilled water and 5 ml of ferric alum as an indicator also added in it. Then after the blended sample was standardized by the addition of 0.1 N KSNW up to the time when the solution color was changed to light brown. After that the salt percentage was determined.

By using titration method [15] titratable amine group of formaldehyde nitrogen was determined. 1 ml of sample was assorted with 9 ml distilled water and titration was done by the pH of 8.3 with 0.1 m NaOH. To become a

blend sample. Total acidity (TA) was calculated & determined by the known volume. The reaction of the amine group was done when we added the 10 ml of formaldehyde solution (38% v/v, pH 9). The reactive nitrogen content FNC of formaldehyde was determined by using the following method.

FNC (g/L) = ml of NaOH (pH 8.3-pH 9) × o.1 × 14

Ammonia Nitrogen Content = Formaldehyde Nitrogen - Ammonia Nitrogen.

Ammonia Nitrogen content was investigated through the process of distillation and the release of volatile Nitrogen into the boric acid using on Buchik 350 distillation reported by the Dissorphong and others [15]. The 5 ml of 10-fold diluted extract from the soy sauce sample was kept in the kjeldal flask having 10 ml distilled water and 3 gm MgO. The moisture was distillated for the release of volatile nitrogen into 40 ml of 4% boric acid containing methyl red bromocresol green for the 5 minutes time. The distillated sample was eventually titrated by 0.05 MH_2SO_4 up to the end. Then after that the end was reached to the ammonia nitrogen content (ANC) was calculated with the following equation.

ANC (g/l) = 5.6× 0.05×10× Y

Based on formaldehyde nitrogen contents and ammonia nitrogen contents ANC follows [16]. Ammonia nitrogen was calculated.

Ammonia nitrogen content (g/L)=Formaldehyde nitrogen content - Ammonia nitrogen content.

Statistical analysis

Package of statistics for social sciences (SPSS version 17) and windows of Microsoft was used for the statistical analysis. By the use Duncan test means were compared & the probability values $P<0.05$ were also be considered the significant one.

Optimization of HPLC conditions

Better separations were achieved in BAs regions under the above-mentioned condition. Figure 1 (A) showed the chromatogram of BAs in standard solution. Eight BAs were well resolved using HPLC method by Uv-vis at 254 nm. Dansyl polyamines were completely eluted from the column after 28 min in tested samples. Individual BAs were identified according to retention time by comparison against standard solutions shown in Figure1 (B) 2.6.

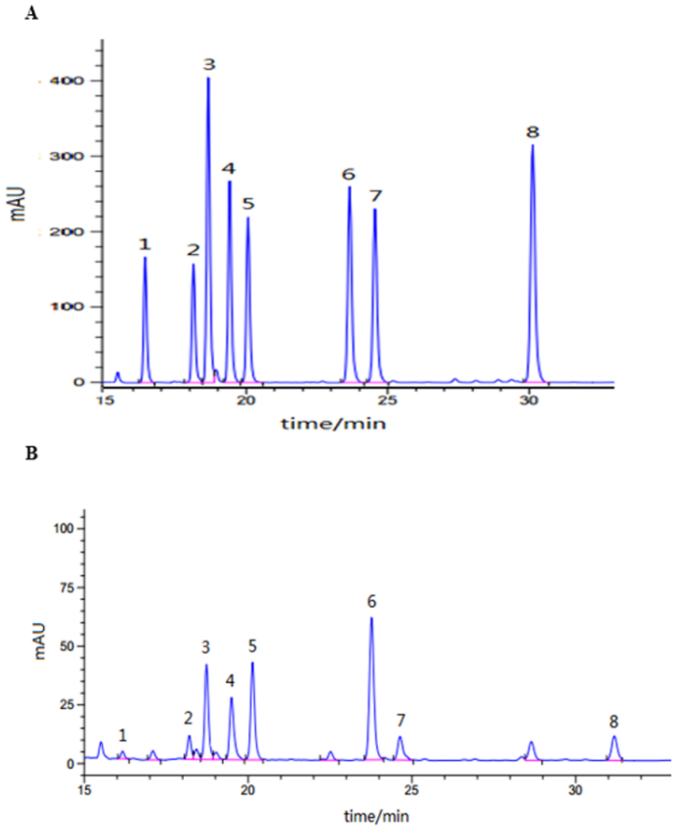

Figure1: (A) Typical HPLC chromatograms of biogenic amines in standard solution (A) and in soy sauce samples, (B)1 - TRP, 2 - 2 - PHE, 3 - PUT, 4 - CAD, 5 - HIS, 6 - TYR, 7 - SPD, 8 – SPM.

Results and Discussion

In the Figure 1(A) chromatogram of BAs was shown as the normal solution. The Bas retention time was consistent, stable and reproduced again. It was shown that the eight BAs were well resolved. With the good resolution and sharpness the different amines were isolated. After 31 min dansyl polyamines were absolutely eluted from the column. Sample analysis of typically chromatogram of Bas in 07TXJY sample was shown in the Figure 1 On the basis of retention

time with the comparison against standard solution were able to know the individual condition of BAs.

In China in the Table 1 the concentration of biogenic amines in the 50 soy sauce were displayed. The tested sample was extremely different in the content of BAs from other kinds of the soy sauce. Out of the 50 samples of soy in the 48 samples spermidine was identified in the samples which are about 96% of the total percent solution. Putrescine was identified 94.00% of the soy sauces samples, Tyramine, Histamine and 2-Phenylethylamine was detected in 88.00%, 82.00%, and 80.00%, respectively, while Cadaverine and Tryptamine was detected in 46.00% and 2.00%, respectively. Spermine was not found in any quantifiable concentration at any samples. Inside the eight biogenic amines samples the total concentration were 3.67 g/L from the minimum to maximum level 0.08 g/L to 16.10 g/L. The highest level of Tyramine was 0-6.22 g/L detected in 50 Chinese soy sauce samples, the following level of BAs was 0-4.43 g/L for Histamine, and the BAs level for each other and the Putrescence was 0-2.18 g/L 0-1.83 g/L for 2-Phenylethylamine, for Cadaverine 0-0.68 g/L for Spermidine 0-0.43 g/L, 0-0.18 g/L for Tryptamine, respectively. In China with in all the soy sauce samples spermines were not detected. The results were in agreement with the previous study wherein 2-phenylethylamine, putrescine, cadavrine and histamine were detected in Korean traditional soy sauces and brewed soy sauces analyzed by the validated HPLC, whereas spermine was not detected in the same aliquots [17]. Tyramine was the dominant BAs in soy sauces, which were strongly, agree with the publications of. The difference between BAs composition and high tyramine content was absolutely in difference from each other which was later on correlated with the high concentration of putrescine is due to the less hygienic conditions during the production time [18].

Ouyang et al., identified that in China with in the soy sauce the amino acid nitrogen (AAN) is one of the extremly important factor which is the main factor for the indication of quality of soy sauce.

Honag et al., identified that during the fermentation of soy sauce the amino acid nitrogen (AAN) should be attributed to the splitting of protease and peptides which cause the hydrolization of protein raw materials in to small peptides [19], ammonia and amino acids. The high quality grade represents that it should be high in the amino acid nitrogen content (AAN) The soy sauce has the higher concentration of amino acid nitrogen (AAN) should have to be good in test. Table 1 displayed the concentration of amino nitrogen of 50 Chinese soy sauce samples, the ranges of concentration were 1.00-2.55 g/L. Amino content in soy sauce should not be less than 0.40 g/L, according to Chinese National Standard GB/T 2717-2003, thus the tested soy sauce products met these standard requirements. However while breaking the standardized ones it will be likely the health risk for the consumer.

In our experimental study 50 soy sauce samples were studied which were further divided in to three groups (Figure 1): low contents (<1.4 g/L, n=18), moderate contents (1.4-1.7 g/L, n=15) and high contents (>1.7 g/L, n=17). In Chinese soy sauces quality grades (good, very good and excellent) were correlated with the intervals of amino acid contents. Inside the tested samples shown in the Figure 2 the total biogenic amines content were generally high but on the other hand the soy sauce samples has low amount of amino nitrogen level and low all biogenic amines contents. Biji, Ravishankar et al. 2016 studied that significant increase amount of biogenic amines were produced during the processing of samples and the storage of the food because of the inadequate storage conditions and the microbial contamination [20]. It was known after the conduction of an experiments that the microorganisms have the enzymatic activity of decarboxylase which convert the amino acids to their associated biogenic amines. Marklinder & Lönner, studied that for the formation of BAs by microorganismsone [21] , the most important prerequisities. Is the availability of free amino acids although not always leading to amine production. The above study shows the fact that there is possible relationship between amino nitrogen level and biogenic amines. Yan et al., studied that in China the fermentation of soy sauce is divided into two types, one is low-salt solid state fermentation (LSF) and the other is high salt liquid state fermentation (HLF). Both the types has the similar four steps principle (1). Raw material preparation (2). Making of Koji (3) Fermentation of Brine (4). Extraction

and blending of sauce. Sano et al., said that among the above steps there is a significant role of the koji making. Inside the Figure 3, total 50 samples of soy sauce were checked for the eight biogenic amines such as spermine [22], spermidine, tyramine, histamine, cadaverine, putrescine, and the average amount of tryptamine, 2-phenylethylamine. By LSF process the concentration of 8 samples were slightly lower than the 42 samples, the difference was observed due to the differences in the manufacturing techniques. At the indigenous fermentation process at 28-35˚C the HLF procedure were runned for the long aging period of time, but by using the pure culture at the high temperature (40˚C -50˚C) in the fermentation process of LSF found the very small amount of aging [23]. By mixing soybeans and wheat flour in the made up of soy sauce in HLF method but in LSF method for the manufacturing of soy sauce soybeans and wheat brans were used Nout et al., reported that the concentrations of cadaverine, putrescine and tyramine which depends on tyramine, in tempeh dependent process of apllied manufacturing which contains soaked beans soybeans [24], type of fermentative microorganisms,boiling, house cooking by in which oil and sweet were added at the same storage temperature conditions (Figure 4).

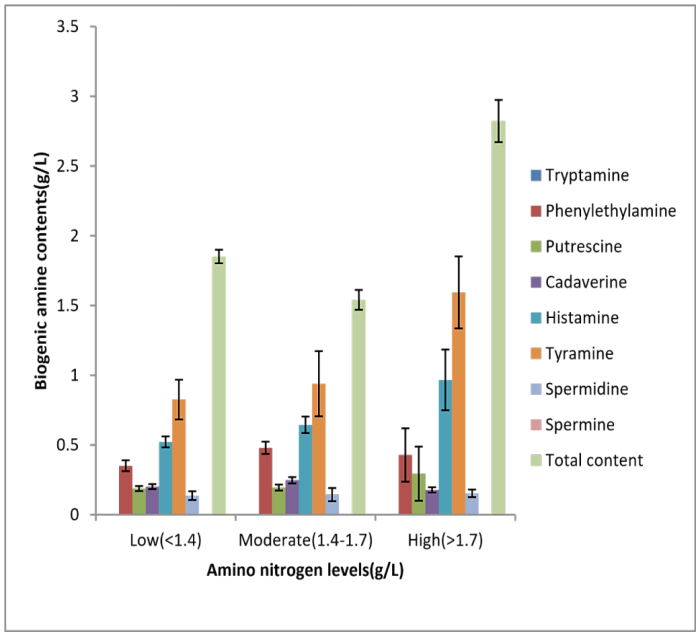

Figure 2: Biogenic amine contents (g/L) versus amino acid nitrogen levels (g/L) in soy sauces. Amino nitrogen levels (g/L): low contents (<1.4 g/L, n=18), moderate contents (1.4-1.7 g/L, n=15) and high contents (>1.7 g/L, n=17).

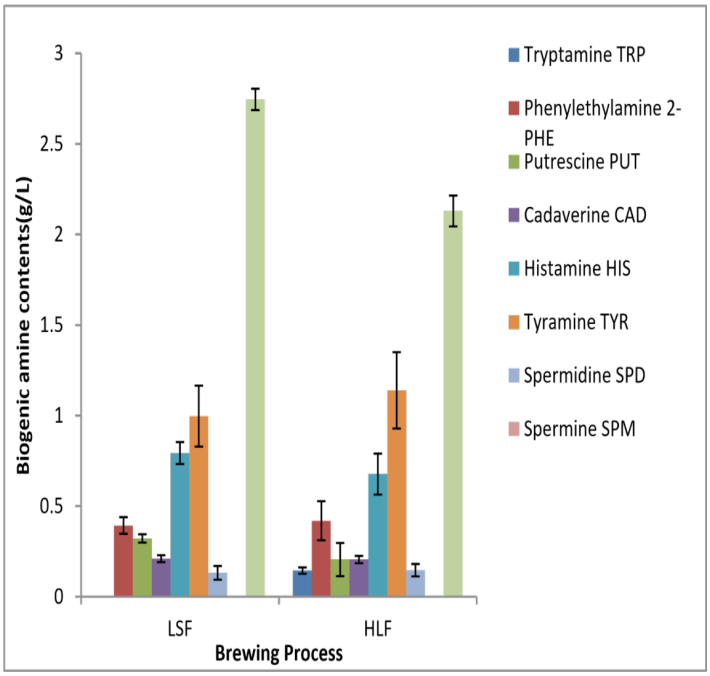

Figure 3: Biogenic amine contents (g/L) in soy sauces produced by different brewing process. HLF, high salt liquid state fermentation (n=42) and LSF, low salt solid state fermentation (n=8).

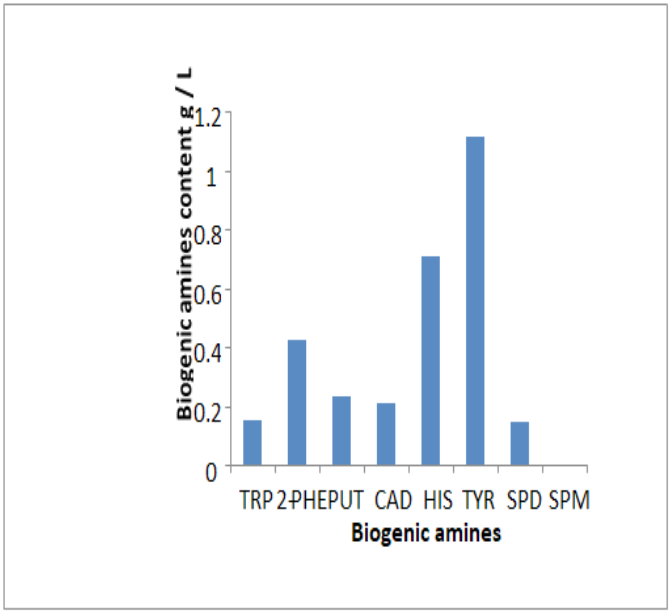

Figure 4: The average amounts (g/L) of different biogenic amines present in the soy sauces
Measured in 50 samples of soy sauce.

BA (tryptamine, 2-phenyl-amine, potricin, cadafirin, histamine, thiamine and aspirin) and mean 0.14 g/L,

0.41g/L, 0.23 g /L, 0.21 g/L. L, 0.70 g/L, 1.11 g/L and 0.15 g/L. Spiramine in all 50 samples. 4, the concentration of thiamine detection in our top 50 of the other BA samples (1.11 g / L), followed by the detection of the content Histamine (0.70 g/l), and at least the quantity of amine. A high level of amine should be indicated as an indicator of microbial contamination, such as lactic acid bacteria [25]. All fermented samples produce soy sauce research concerning lactic fermentation, which may involve amines. BA thiamin as a dominant bacteria is attributed to the large amount of thiamine transformation capacity. Because we do not know the source and historical tradition, therefore, it may be due to the source, treatment conditions and extent of microbial contamination changes in BA levels. The following studies have shown that the factors of production, including the conditions of BAS [26] and the manufacturing process [27]. Allah (Rialtta MAIJALA, EEROLA, LIEVONEN) (Free amino acids, HILL, and Hervi) [28], and relative abundance [29].

Bioamines have been widely studied in foods, and polyamines, such as potrescine, cadaverine, spermine and spermidine, are naturally present in food and are involved in cell growth and proliferation. However, the appearance of these amines is also associated with the presence of nitrite, which can potentially become carcinogens when converted to nitrosamine [12]. Bacteria are anti-natural and important factors from a healthy point of view, because they can, as pathogens, influence a number of food poisoning episodes, and they are able to initiate various drug interactions. Nitrosamine polyamines may not necessarily pose a health hazard, reaching toxic concentrations only after consuming large quantities, which is more than expected in daily food. It has been reported that aromatic BA, including tyramine and 2-phenylethylamine, are initiators of a diet and hypertension caused by migraine [30]. Tramin, 2-phenylthylamine and potricin are effective vasodilators and increase blood pressure, which can lead to heart failure or bleeding in the brain [31]. And anti-tertiary anti-nutritional complexes. However, it is very difficult to determine the threshold for the exact toxicity of a bachelor in individuals. According to Nout [32], consuming more than 0.04 g ba for each meal is considered toxic.

In addition, other studies [33, 34] show that histamine with a concentration of 0.50 g / kg in food can be dangerous for human health. On the other hand, Ten et al., [35] reported that 0.10-0.80 g/kg of tyramine in food is toxic. Santos suggested that more than 1 g/kg (the total amount of amine in food) is dangerous for hygiene [29]. As shown in Table 3, 38.00% of the soy sauce samples (n=19) showed that the histamine content exceeds 0.5 g/kg, which is higher than the values that are considered hazardous to human health, and the histamine toxicity is It appears to be enhanced by other amines, such as Gadafirin, Potricin, and Tyramine.

Table 3: Distribution of histamine, tyramine and total biogenic amine contents (g/L) in the 50 tested soy sauces.

Biogenic amine contents (g/L)	Number of soy sauce samples		
	Histamine	Tyramine	Total
<0.099	3	2	2
0.1-0.249	6	6	5
0.25-0.499	13	6	3
0.5-0.999	7	10	5
>1	12	20	35

It is reported that only histamine cannot cause low-level toxicity and no toxicity. Other BS, such as potricin and cadavirin, are 5 times higher than histamine alone, can increase the toxicity of histamine by inhibiting histamine oxidation enzymes. The oral toxicity levels of botarin, alcohols and spiramidine are 2, 0.6 and 0.6 g/l, respectively [36]. According to Table 1, the content of these amines in our study did not exceed the standard in every 50 samples of soy sauce. Only 2 soy sauce (01JY, 01LCW) showed tyramine content less than 0.1 g/kg. The concentration values of 48 other samples were higher than 0.1 g/kg, which was considered toxic from table 3. Soy sauce samples (n=35) make up more than 1 g/kg, which is higher than the levels considered dangerous to fitness. Our results show that a high level of BS in soy sauce can accelerate the syndrome of a Chinese restaurant to some extent. People suffer from these syndromes, which can result from eating a high lev-

el of bioamine for every meal, especially when people eat meat with sausage, rice, cheese, or other fermented foods. More rigorous research is needed to determine all the causes of this syndrome.

It is known that pH plays an important role in the development of soybean fermentation. Bacteria are formed by removing the enzymatic carboxylic acid from amino acids, whereas pH is a factor that affects the activity of aminodeproxylase, since the activity of dicarboxylic remains intact in an acidic medium [29]. Thus the level is very important for the content of the bachelor. ZaMaN found significant correlations between pH, salt content, number of air trays, BS content and BS levels in fish broths, which depend on relevant variables such as pH. Values and salt content, and found that the optimum pH ranged from 4.0 to 5.5 in soy sauce. Santos also reported that a low pH of around 3.0-6.0 was ideal for bacteria to express decarboxylase [29]. The pH values for 50 soy sauce samples in China are shown in Table 2. We found that the pH levels for all samples were between 4.0 and 6.0, and these results were largely consistent with previous results [30, 13]. In addition, low pH levels of soy sauce samples may increase the activity of decarboxylase [37, 38].

Sodium chloride is one of the most important components of many products. This affects not only the food profile, but also its texture, and plays a vital role in supporting food against microbes [39]. Suo et al., reported that a combination of 5% sodium chloride and fermentation at 45° C was the best example of Koji extraction for soy sauce [40]. However, it is reported that Nacl increases the risk of developing hypertension and is directly related to the development of cardiovascular diseases. Therefore, in 2007, WHO recommended limiting the average intake of NaCl to 5 g per day for adults (WHO 2007). According to the Chinese food and temple guidelines of the Pagoda Food Guide 2016, the average consumption of sodium chloride is 6 g per day for adults. Table 1 show that the concentration of sodium chloride in 50 samples of soy sauce in China was 15.16 g/100 ml in the range from 10.21 to 21.68 g/100 ml. Because the average content of sodium chloride is approximately 2.27 grams per day, while the average intake of soy sauce is 15 ml per day for the Chinese. Thus, the concentration of sodium chloride in 50 Chinese soy sauce meets the criteria. In soy sauce products, a high salt concentration is appropriate and causes high osmotic pressure, which allows for exceptional development of sensory properties and preservation [41]. However, long-term defects in brewing include an increased risk, such as hypertension, as well as being directly related to the development of cardiovascular diseases [19]. Thus, reducing the salt content in soy sauce has been a recent effort and has become an important issue for the food industry.

Formaldehyde nitrogen is an indicator of the degree of protein degradation in soy sauce. As a rule, it is measured by a voltage measurement method in which formaldehyde reacts with amino acids and releases 1 H + ion from the amino group [42]. The change in FNC was shown in the water fraction 50 samples of soy sauce in China in table 1, and the concentration of FNC was 5.71-18.44 g/ l, the lowest FNC was detected in the sample (03HDSC), which indicates a slow hydrolysis of protein soy protein. These data are consistent with the previous report [43]. The increase in nitrogen content in formaldehyde was associated with increased peptide hydrolysis caused by microbial or microbial proteins. In addition, a smaller amount of FNC was found in samples with a higher salt content and lower pH, which indicates that the formation of FNC may be mediated and associated with changes in pH and salt content, which is consistent with other results [44-51]. The decomposition of proteins into small peptides will contribute to taste, especially the development of taste.

Conclusion

In conclusion, the bioamines content of origin was completely different in samples of Chinese soybeans. Spermidine, putrescine, tyramine, histamine and 2-phenylthylamine were the most common bio-amines in Chinese soy sauces. However, there were significant differences in the content of bioamine in soy sauce at different levels of amino-containing amino acids. Contrast to 8 levels of bio enzymes from 50 samples with a change in pH, salt content and formaldehyde content. This may be due to the production technology and

the type of fermentation. Overall, a total of eight amino acids were high in the samples, which contained high levels of amino nitrogen.

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Authors contribution

Muhammad Altaf Hussain and Muhammad Israr khan Shiyue Pang contributed to the writing of the manuscript. sha Changbao Sun[a], Zhijing Liu[a], made the figures and tables. Feng Xue and Lianzhou Jiang analyze the data. Zhanmei Jiang and Juncai Hou designed the plan.

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