Occurrence of biogenic amines in Thai soy sauces and soy bean pastes and their health concern

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Abstract

Biogenic amines (BAS) in 90 samples with different brands and formula of Thai fermented soy sauces (SS) and soy bean pastes (SBP) were analyzed by using HPLC after pre-column derivatization with dansyl chloride. Our results show that all SS and SBP samples contained BAS ranging from 2.10 to 594.60 ppm and from 3.21 to 82.18 ppm respectively. The type and the levels of BAS in the products varied depending on brands and formula. Of the 7 BAS studied, putrecine and cadaverine were the most common found (100% occurrence) in the SS samples followed by tyramine (93.33%) and histamine (86.67%). For SBP, occurrence of putrecine, cadaverine and spermidine (100%) were the most prevalent found in all samples, following by tyramine (85.71%). Our study revealed correlation between total BAS and some types of BAS including histamine putrecine and tyramine (p<0.01). The BAS levels in SS and SBP studied were mostly lower than the values considered harmful to consumer health.

Introduction

Biogenic amines (BAS), low-molecular-weight nitrogenous organic molecules, are mainly generated in foodstuffs by various groups of microorganisms capable to decarboxylate amino acids such as lactic acid bacteria, yeast and Enterobacteriaceae (Shalaby, 1996; Önal, 2007). BAS are found in varieties of food products especially fermented foods including fish, fruit juices, cheese, wine, vinegar and meat products (Önal, 2007; Saaid *et al*., 2009; OrdÓñez *et al*., 2013). Histamine (HIS), putrescine (PUT), cadaverine (CAD), tyramine (TYR), 2-phenylethylamine (PHE), tryptamine (TRYP) and spermidine (SPD) are the most common BAS found in foods (Shalaby, 1996; Saaid *et al*., 2009). Main factors contributing to the types of BAS and their accumulations in foodstuffs are available free amino acids and presence of decarboxylating microorganisms. Moreover critical concentrations of BAS are also relevant to the microbial contaminations (Halász *et al*., 1994). Once BAS are formed, it is difficult to completely remove from the products even with high temperature treatments (Bai *et al*., 2013).

Consumption of BAS at low concentration in average diets is not regarded as a serious health risk. On the other hand, if the amount consumed reaches threshold or general catabolism of BAS is inhibited; various physiological effects such as headache, diarrhea, nausea, red rash, high blood pressure, cardiac palpitation and death in severe cases may occur as the consequence (Halàsz *et al*., 1994; Shalaby, 1996; Romero *et al*., 2000). Considering toxicity of biogenic amines, HIS, TYR and PHE are of major concern (Shalaby, 1996). HIS induces various symptoms in respiratory tract, cardiovascular system, central nervous systems and skin (Maintz and Novak, 2007). While TYR and PHE induce hypertension and migraine (Shalaby, 1996), PUT and CAD are known to enhance histamine toxicity by inhibiting HIS detoxifying enzymes (Lehane and Olley, 2000). In addition, CAD and PUT can be precursors for formation of carcinogenic nitrosamines (Önal, 2007). Hence, monitoring the levels of BAS in foods is crucial not only for safety concern, but also for an indicator of food freshness.

Fermented soy sauce (SS) and soy bean paste (SBP) are the most popular Asian seasoning products which are consumed and produced worldwide today. They have been used as condiments to improve flavour and taste of foods. Beside they are also regarded as salt substitutes and sources of protein and antioxidant (Stute *et al*., 2002; Zhu *et al*., 2010; Yang *et al*., 2011). Traditional fermented SS and

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SBP are usually produced simultaneously from a mixture of cooked prime soybean with wheat flour allowed to ferment for 40 to 48 h by koji mould (i.e. *Aspergillus sojae* or *Aspergillus oryzae*). The growing mould on soybean and flour that is so-called "koji" produces enzymes to hydrolyse substrates such as protein and polysaccharide. The koji is then immersed in a brine solution to obtain "moromi" in which lactic acid bacteria and yeast are responsible for lowering of the pH and developing organoleptic properties (Mongkolwai *et al*., 1997; Feng *et al*., 2015). After a period of moromi fermentation time (1-10 months), the moromi mash is then pressed to separate liquid part and residue solid portion. These are then pasteurized and bottled to obtain the SS and SBP respectively (Mongkolwai *et al*., 1997; Zhao *et al*., 2011). In Thailand, some fermented SS can also be prepared as a blend of traditional brewed SS derived from the repeated fermentation processes. However, the manufacturing techniques of fermented SS and SBP products can be different depending on manufacturers and traditions of origin countries (Feng *et al*., 2015).

Occurrence of BAS in fermented SS and SBP has been addressed in several surveys, especially those produced in Asian countries such as China, Korean and Malaysia (Stute *et al*., 2002; Kung *et al*., 2007; Yongmei *et al*., 2009; Saaid *et al*., 2009; Shukla *et al*., 2010; Guidi and Abreu-Gloria, 2012). Total BAS in fermented SS products were found in levels ranging from 2 up to 6000 ppm with HIS, TYR and TRYP were predominant (Stute *et al*., 2002; Yongmei *et al*., 2009; Saaid *et al*., 2009). In addition, total BAS in fermented SBP products were found in the range of less than 50 to 7250 ppm, in which PUT, SPD, TYR were dominant (Kung *et al*., 2007; Shukla *et al*., 2010; Shukla *et al*., 2014). Up to now, many studies have demonstrated the presence of BAS in fermented SS and SBP samples from various countries; limited information is available concerning the products produced in Thailand (Stute *et al*., 2002). Especially, there were few reports studied on the amount of BAS in SBP products. The objective of this work was to investigate the amount of BAS in Thai fermented SS and SBP products. Moreover, chemical and microbiological properties including pH, salt content, amino acid nitrogen, total bacteria and total yeast mould were also analysed.

Materials and Methods

Chemicals and standard preparations

Biogenic amines standards including putrescine dihydrochloride (PUT), histamine dihydrochloride (HIS), cadaverine dihydrochloride (CAD), 2-phenyl-ethylamine (PHE) and spermidine trihydrochloride (SPD) were purchased from Sigma-Aldrich (USA). Tryptamine hydrochlorides (TRYP), Tyramine hydrochloride (TYR) and dansyl chloride (derivatising reagent) were obtained from Fluka (Swizerland). 1,7-Diaminoheptane and acetone were from Merck (Germany). HPLC-grade acetonitrile was from Mallinkort (USA). Ultrapure water was generated using a Milli-Q purification system (Millipore, Bedford, MA, USA). All other chemicals were of analytical grade. Stock solution containing a mixture of those amines (500 ppm) was prepared in a 0.4 M perchloric acid and stored in the dark at 4°C until used. Standard solutions were prepared by diluting the stock solution and used to construct calibration curves.

Thai fermented SS and SBP samples

Ninety bottles of Thai SS covering fifteen brands with formula 1-5, and eight brands of Thai SBP with formula 1 and 2 (three bottles per brand and per formula, the same production batch) were purchased from supermarkets in Thailand between 2010 and 2013. The formula of these products was categorized based on their manufacturers' recipes. Briefly, formula 1 is produced by using good quality soybean and wheat flour as raw materials in fermentation process where different ratios of soy beans, wheat flour, and salt brine solution are varied based on manufactures' recipe. In case of soy sauce, moromi solid residue obtained after draining raw soy sauce can be repeatedly added with salt brine to conduct lower quality of moromi fermentation. Formula 2 is prepared by blending the SS formula 1 with raw soy sauce produced from the first repeated fermentation of moromi solid residue. This repeating fermentation can be accomplished up to four times. Formula 3, 4, and 5 are produced by blending the SS formula 1 as a base and added with different ratios of SS formula 2 and the raw soy sauce produced from repeated fermentations of moromi mash.

For soybean paste, the formula 1 and 2 are prepared in the same manners but less percentage of soy bean used in the formula 2. All bottles were coded and stored under cool conditions without direct sunlight until analysis. Each sample was opened immediately prior to BAS, chemical and microbiological assays.

Sample preparation

The samples of SS and SBP were extracted according to the method of Shukla *et al*. (2010) with some modifications. A 5-ml portion of SS was mixed with 25 ml of 0.4 M perchloric acid containing a

Table 1. Analytical characteristics of the biogenic amines method using for soy sauce (SS) and soy bean paste (SBP)

BAS	R[b]		LOD[c]		LOQ[d]		RSD[d]		Recovery	
	SS	SBP	SS	SBP	SS	SBP	SS	SBP	SS	SBP
			(ppm)	(ppm)	(ppm)	(ppm)	(mv[f],%, n =10)	(mv,%, n =10)	mv, %, n =8)	(mv, %, n =8)
TRYP[a]	0.98	0.99	0.038	0.030	0.130	0.090	5.17	6.00	96	101
PHE	0.97	0.98	0.015	0.125	0.050	0.416	5.33	6.90	89	92
PUT	0.99	0.99	0.015	0.038	0.050	0.125	4.18	5.97	88	95
CAD	0.99	0.99	0.038	0.075	0.127	0.250	5.77	3.87	95	93
HIS	0.99	0.99	0.005	0.019	0.016	0.063	5.70	4.00	96	90
TYR	0.99	0.98	0.038	0.125	0.127	0.417	4.82	6.33	99	96
SPD	0.96	0.98	0.017	0.075	0.056	0.250	9.49	6.83	95	98

[a]TRYP, Tryptamine; PHE, 2-Phenylethylamine; PUT, Putrecine; CAD, Cadaverine; HIS, Histamine; TYR, Tyramine; SPD,Spermidine

[b]R, Regression coefficient; [c]LOD, Limit of detection; [d]LOQ, Limit of quantification; [e]RSD, Relative standard deviation; [f]mv, mean value

known amount of 1,7–diaminoheptane as internal standard for 5 min; then filtered through a filter paper (Whatman No. 1, Maidstone, England). For SBP, a 5-gram ground sample was suspended in 25 ml 0.4 M perchloric acid containing a known amount of an internal standard. The mixture was homogenized for 5 min and then the supernatant was collected by using a centrifugation (Sigma model 2-16 PK, Sartorius, England) at 1000 x g, 4°C for 20 min; then filtered through a filter paper (Whatman No. 1, Maidstone, England). One milliter of each extract was then derivatised with dansyl chloride prior to HPLC analysis.

Derivatisation procedure

Derivatisation of BAS was carried out according to Saaid *et al.* (2009) with some modifications. One milliter of the sample was mixed with 200 μl of 2 M sodium hydroxide, 300 μl of saturated sodium hydrogen carbonate and 1 ml of dansyl chloride solution (10 mg of dansyl chloride in 1 ml acetone). The mixture was vortexed and then heated at 70°C for 10 min. One hundred microlitre of 30% ammonium hydroxide solution was added to stop the reaction and to remove residual dansyl chloride. After leaving at room temperature for 30 min, the mixture was then adjusted to 5 ml with acetonitrile and filtered through a Teflon membrane filter (pore size 0.45 μm, Millipore).

Separation of biogenic amines by HPLC

Analysis of BAS was performed using HPLC equipped with a diode array detector (Agilent 1100, USA) set at 256 nm. The sample was separated on a C_{18} column (150 × 4.6 mm, 5 μm, Zorbax Eclipse Plus) with a C_8 guard column (12.5 × 4.6 mm, 5 μm, Zorbax C8). A gradient elution system with a mixture of water as solvent A and with acetonitrile as solvent B was used. The gradient started at 35% A at 0 min, 30% A at 5 min, 0% A at 20 min and 35% A at 25 min. BAS were quantified based on peak areas corresponded to retention times of derivatised BAS standards.

Chemical analysis

The NaCl content was determined according to Volhard's method (AOAC, 2000). Amino nitrogen content was carried out by using the titration procedure as described by Shively and Henick-Kling, 2001. The pH of SS was measured directly using a pH meter (IQ Scientific Instrument, USA). For the pH measurement of SBP, 25 ml of deionized water adjusted to pH 7 was added to a 5-g ground sample and homogenized with an Ultra Turrax at 6000 rpm for 3 min. After filtration through a filter paper (Whatman No.1, Maidstone, England), the pH of the filtrate was then determined.
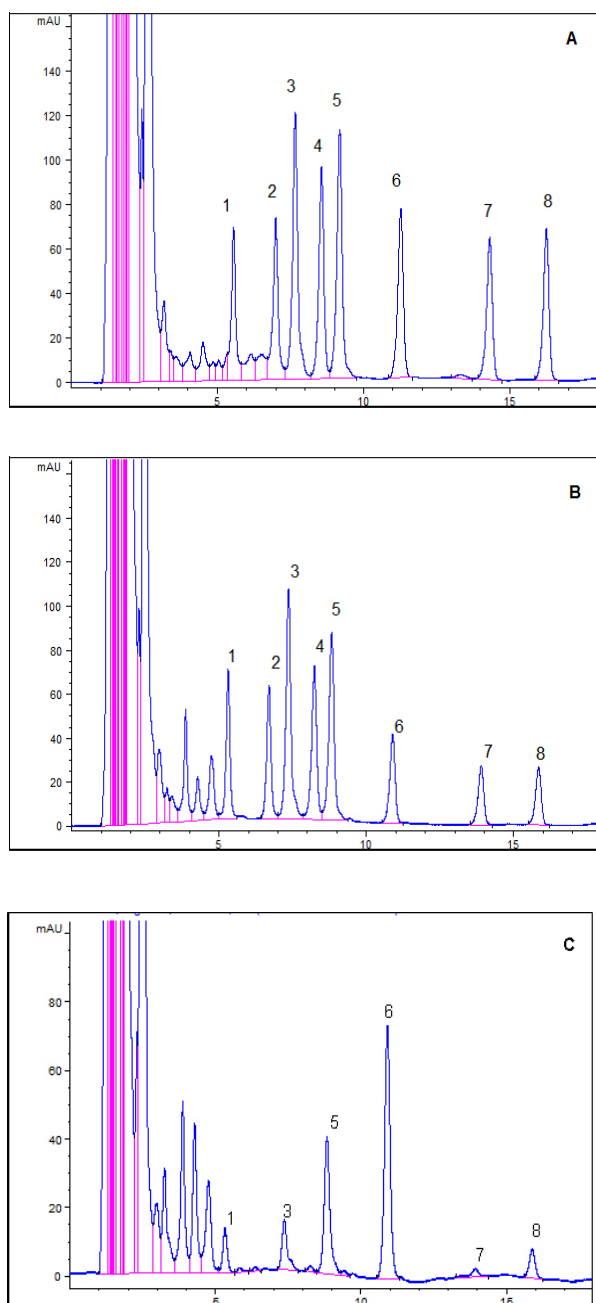
Figure1. Typical HPLC chromatogram of standard biogenic amines (A); HPLC chromatogram of a soy sauce sample (B); HPLC chromatogram of a soybean paste sample (C). Peak identification: 1, Tryptamine; 2, 2-Phenylethylamine; 3, Putrecine; 4, Cadaverine; 5, Histamine; 6, 1,7-diaminoheptane; 7, Tyramine; 8, Spermidine.

Microbiological analysis

A 10 ml of SS was transferred into a sterile tube containing 90 ml of sterile saline solution (9 g l-1 NaCl) and mixed with a vortex mixer for 3 min. For SBP, 10 g of sample was taken into a sterile container containing 90 ml of sterile saline solution (9 g l^{-1} NaCl) and homogenized with an Ultra Turrax at 6000 rpm for 3 min. Analysis of viable cell counts was performed according to a standard aerobic plat

count procedure. Bacteria and yeast-mould counts were carried out on brain heart infusion agar (BHI, containing 10% NaCl and a fungicide amphotericin B 50 mg l^{-1}) and Chloramphenicol Glucose Agar (Biokar) with 10% NaCl, respectively. Agar plates were incubated for 2-3 days at 37°C for bacteria and 30°C for yeast and mould.

Statistical analysis

Each type of sample (n=3) was analysed in triplicate. Analysis of Pearson correlation was performed to determine the relationship between the parameters of interest (p<0.01), using the XLSTAT PRO 2012 statistical package for Windows (Addinsoft, New York, USA).

Results and Discussion

Method performance for BAS analysis

The BAS analytical method was validated based on linearity, limit of detection (LOD), limit of quantification (LOQ), recovery and repeatability. Standard addition method was applied in this study to overcome matrix effects of both SS and SBP on BAS determinations. Results are summarised in Table 1. Linear regression analysis was carried out with standard curves constructed with six concentration levels (0.5-40 ppm) in triplicate, showing good linearity (r = 0.96-0.99). LOD was determined from the minimum concentration required to give a signal-to-noise ratio of 3; while LOQ was estimated with a signal-to-noise ratio of 10. The LOD of each BAS in SS and SBP were in ranges of 0.005-0.038 ppm and 0.019-0.125 ppm, respectively. The LOQ of each BAS in SS and SBP were in ranges of 0.016-0.130 ppm and 0.063-0.417 ppm. Repeatability in term of relative standard deviations (RSD, n =10) of each BAS in both SS and SBP were between 3.87-9.49%. Recovery studies were performed with eight replicates by spiking known samples with a mixture of BAS standard at concentration 5 ppm for SS and SBP. Acceptable recovery obtained for all BAS in both SS and SBP was 88 to 101%. These are comparable to those reported in the literature (Stute *et al.*, 2002; Saaid *et al.*, 2009). The methods validated for determining BAS were therefore applied to the SS and SBP products. An internal standard method was used to determine the BAS contents. The HPLC chromatogram for standard BAS and that for SS and SBP samples are given in Figure 1.

Occurrence of BAS in SS samples

The seven BAS of interest were detected in all Thai fermented SS products as shown in Table 2.

Table 2. Type and level of biogenic amines found in Thai fermented soy sauce (SS) and soy bean paste (SBP) samples

Products	Positive samples/ Total		Concentration (ppm)							
			TRYP[a]	PHE	PUT	CAD	HIS	TYR	SPD	Total
SS Formula 1	45/45 (100%)	Range	n.d.[b]-6.40	n.d. - 38.00	0.30-229.00	0.33-14.00	n.d-153.00	n.d. -166.00	n.d -10.40	2.34 -594.60
		Mean	3.80	5.99	22.99	3.44	42.97	31.01	3.99	99.18
		SD	1.85	11.71	60.25	3.81	53.80	46.88	3.25	157.33
SS Formula 2-5	18/18 (100%)	Range	n.d.	n.d-7.00	0.34-97.00	n.d.-2.90	n.d.-98.00	n.d. -128.00	n.d. -4.20	2.10-332.90
		Mean	n.c.	3.70	16.76	0.98	19.06	26.78	2.26	61.85
		SD	n.c.	4.67	39.31	1.08	38.76	56.58	1.46	132.89
SBP Formula 1	24/24 (100%)	Range	n.d-2.77	n.d -1.87	0.33-3.39	0.33-23.83	n.d-13.82	n.d-38.28	<LOQ[c] - 9.35	3.21-82.18
		Mean	1.17	0.25	1.25	3.49	4.14	5.81	3.97	19.23
		SD	0.97	0.66	1.01	8.22	5.12	13.15	2.90	24.33
SBP Formula 2	3/3 (100%)	Range	3.03	n.d.	1.12	0.36	0.54	1.68	5.45	12.18
		Mean	n.c.[e]	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
		SD[d]	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.

[a] TRYP, Tryptamine; PHE, 2-Phenylethylamine; PUT, Putrecine; CAD, Cadaverine; HIS, Histamine; TYR, Tyramine; SPD, Spermidine

[b]n.d., refers to below than the limit if quantification; [c] < LOQ, refers to below than the limit of quantification; [d] SD, standard deviation; [e] n.c., not calculated

No bottle variation in types of BAS was found in this study. The type and the concentration of BAS in the samples varied depending on brand and formula. Among the 15 brands of SS studied, the highest amount of BAS was found at 594.60 ppm. Concerning variation due to the formula, the SS formula 1 showed more types and higher amount of BAS than those of the rest of the formulas. PUT and CAD were the most common BAS detected (100% occurrence) in the samples of formula 1, followed by TYR (93.33%) > HIS (86.67%) > SPD (80%) > PHE (73.33%) > TRYP (46.67%). The levels of seven BAS were less than 6.40 ppm for TRYP, less than 38.00 ppm for PHE, 0.30-229.00 ppm for PUT, 0.33-14.00 ppm for CAD, less than 153.00 ppm for HIS, less than 166.00 ppm for TYR, and less than 10.40 ppm for SPD. In SS samples of the formulas 2, 3, 4, and 5, PUT was detected in all samples (100%), followed by CAD, HIS, TYR, SPD (88%) and PHE (33.33%). No TRYP was found in any samples studied. The BAS level ranged from less than 7 ppm for PHE, 0.34-97.00 ppm for PUT, less than 2.90 ppm for CAD, less than 98.00 ppm for HIS, less than 128.00 ppm for TYR, and less than 4.20 ppm for SPD. The presence of HIS, TYR and CAD was usually associated with microbial contamination during the fermentation process (Santos 1996; Shalaby 1996; Önal, 2007). SPD is generally carried over from raw material with plant origins, while PUT is synthesized by both vegetable tissues and microorganisms including contaminants and starter cultures added (Önal, 2007; Wunderlichová et al., 2014). According to Stute et

al. (2002), TYR is formed at the beginning of the fermentation through a microbial contamination by Enterococcus faecum.

The average amount of BAS in the SS of formula 1 being much higher than those of the formula 2-5 was probably due to some variations in the manufacturing process such as quality and ratio of the raw material used, microbial population, and conditions of fermentation (Shalaby, 1996; Yongmei et al., 2009). The types and levels of BAS in all samples studied were substantially similar to those reported in previous surveys ranging between 2 ppm to 6000 ppm (Stute et al., 2002; Yongmei et al., 2009; Saaid et al., 2009; Guidi and Abreu-Gloria, 2012). Similar to Thai SS, interestingly TYR followed by SPD and HIS were the most prevailing amines in Chinese fermented SS, with total concentrations in the 41.7-1358 ppm range (Yongmei et al., 2009). In Brazilian SS products, TYR (3.00-659.9 ppm) was the most found BAS followed by PUT (less than 180.0 ppm) and HIS (less than 395 ppm) in succession (Guidi and Abreu-Gloria, 2012).

Chemical and microbiological analysis of SS samples

As shown in in Table 3, no large brand and formula variations in pH and NaCl content were observed in this study. The pH and NaCl content in the samples ranged from 4.14 to 4.78 and 12.41% to 22% respectively. These results are corresponded to previous reports from various countries (Stute et al., 2002; Yongmei et al., 2009). On the other hand, the

Table 3. The values of pH, % NaCl and amino acid nitrogen of Thai fermented soy sauces (SS) and soy bean paste (SBP) samples

Products			pH	% NaCl	Amino acid nitrogen (ppm)
SS Formula 1	(n = 45)	Range	4.14-4.78	12.98-21.16	851.67-5606.42
		Mean	4.54	16.72	3222.77
		SD[a]	0.20	2.54	1476.77
SS Formula 2-5	(n = 18)	Range	4.27-4.62	12.41-22.00	1502.67-3340.75
		Mean	4.62	19.05	2254.35
		SD	1.22	3.91	643.73
SBP Formula 1	(n = 24)	Range	4.01-4.97	5.77-14.30	1108.33-2872.92
		Mean	4.54	10.63	2120.78
		SD	0.31	2.98	658.96
SBP Formula 2	(n = 3)	Range	4.43	14.40	1309.58
		Mean	n.c.	n.c.	n.c.
		SD	n.c.	n.c.	n.c.

[a]SD, standard deviation; bn.c., not calculated

Table 4. Pearson's Correlation coefficients, R, for relationships between parameters

	TRYP[a]	PHE	PUT	CAD	HIS	TYR	SPD	BAS
TRYP	**1**	0.063	0.013	0.215	0.415	0.156	0.202	0.220
PHE	0.063	**1**	0.971 [b]	0.296	**0.717**	**0.864**	-0.269	**0.915**
PUT	0.013	**0.971**	**1**	0.222	**0.704**	**0.915**	-0.294	**0.931**
CAD	0.215	0.296	0.222	**1**	**0.467**	0.402	-0.202	0.412
HIS	0.415	**0.717**	**0.704**	0.467	**1**	**0.848**	-0.150	**0.906**
TYR	0.156	**0.864**	**0.915**	0.402	**0.848**	**1**	-0.340	**0.976**
SPD	0.202	-0.269	-0.294	-0.202	-0.150	-0.340	**1**	-0.257
BAS	0.220	**0.915**	**0.931**	0.412	**0.906**	**0.976**	-0.257	**1**

[a] TRYP,Tryptamine; PHE, 2-Phenylethylamine; PUT, Putrecine; CAD,Cadaverine; HIS, Histamine; TYR, Tyramine; SPD, Spermidine; BAS, Total biogenic amines

[b] Values in bold are significant at p <0.01

amino acid nitrogen contents widely distributed from 857.61 ppm to 5606.42 ppm in Thai SS products depending on brand and formula. The SS formula 1 had higher amino acid nitrogen content (3222.77 ppm) comparing to other formulas (2254.35 ppm). Among 15 brands studied, the highest amino acid nitrogen content was found at 5606.42 ppm. For microbiological analysis, total yeast and mould of all Thai SS sold were less than 10 CFU/ ml. Total

bacteria of the finished products were mostly less than 100 CFU/ ml, except two samples of brand SS 13 and SS15 had 9.3×10^4 CFU/ ml and 1.1×10^5 CFU/ ml.

Occurrence of BAS in SBP samples

The BAS content in SBP samples are shown in Table 2. No great bottle variation in BAS content was observed in this study. All Thai fermented SBP

samples were found to be contaminated with BAS at varying levels depending on brand and formula. Among the 8 brands studied, the highest BAS content reached 82.18 ppm. The SBP formula 1 showed the greater amounts of BAS than that of the formula 2. The most prevalent BAS in SBP formula 1 were PUT, CAD, and SPD (100%) followed by TYR (85.71%), >TRYP (71.43%), HIS (71.73%) and PHE presented only 25% of the samples. The concentrations of seven BAS were less than 2.77 ppm for TRYP, less than 1.87 ppm for PHE, 0.33-3.39 ppm for PUT, 0.33-23.83 ppm for CAD, 0.29-13.82 ppm for HIS, less than 38.28 ppm for TYR and 0.99-9.35 ppm for SPD. The concentrations of BAS was 3.03 ppm for TRYP, 1.12 ppm for PUT, 0.36 ppm for CAD, 0.54 ppm for HIS, 1.68 ppm for TYR and 5.45 ppm for SPD. PHE was not detected in the sample analysed. In general, the total BAS concentrations of Thai fermented SBP samples were in the 3.21- 82.18 ppm range.

The BAS levels accumulated in the SBP depended on the techniques of production including soaking soybean, types of fermentative microorganism, boiling or cooking and the storage temperature (Nout *et al*., 1993). Similar occurrences of these amines in fermented SBP products have been previously reported with total BAS contents in the range of less than 50 ppm to 7250 ppm (Kung *et al*., 2007; Shukla *et al*., 2010; Shukla *et al*., 2014). TYR, SPD, PUT and HIS were the most BAS found in Korean fermented SBP products (Shukla *et al*., 2010; Shukla *et al*., 2014). Very few data of BAS contamination in fermented SBP have been reported until now. Only the reports on BAS in Taiwan and Korean fermented SBP have been available. The results also reveal that the average concentrations of BAS in Thai SBP were much lower than in Thai SS. Some studies showed that the contaminations of BAS in the SS and SBP products were raised mainly by bacterial contaminations including strains from the genera *Enterococcus*, *Bacillus* and *Staphylococcus* (Kirschbuam *et al*., 2000; Kung *et al*., 2007).

Chemical and microbiological analyses of SBP samples

As shown in Table 3, the pH, the salt content and amino acid nitrogen content values were from 4.01 to 4.97, from 5.77% to 14.40%, and from 1108.33 ppm to 2872.92 ppm, respectively. For microbiological analysis, total yeast and mould of all Thai commercial fermented SBP samples had total yeast and mould < 10 CFU/ g and total bacteria< 100 CFU/ g, apart from the sample SBP 8 had 9.6×10^4 CFU/ g.

Statistical analysis

Pearson's correlation coefficients among variables were shown in Table 4. No significant correlation (p<0.01) was observed between the BAS contents and other parameters investigated including pH, NaCl content, amino acid nitrogen and total microorganisms in the final products. Indeed, the amount and types of BAS formed in foods is a complex phenomenon, depending on several variables such as raw materials, processing conditions, growth kinetic microorganisms, their proteolytic and decarboxylase activities, and their interaction among others (Gardini *et al*., 2001; Carelli *et al*., 2007). However, significant correlation coefficients were found among types of BAS (p<0.01). Especially PHE correlated with PUT, HIS and TYR with r =0.971, 0.717, 0.864, respectively. Moreover, HIS correlated with TYR (r =0.848). These results are in line with the reported previously (Guidi and Abreu-Gloria, 2012). Interestingly, based on strong correlations between total BAS and each of PHE, PUT, HIS, and TYR, these BAS reflect the total BAS. Hence, they can possibly be used as a single parameter for monitoring process of BAS in the products. This needs the further investigation.

Potential Health effects of Thai fermented SS and SBP with BAS

The BAS toxic threshold levels in human are difficult to define since they depend on individual responses and the presence of other amines (Ten-Brink *et al*., 1990; Halàsz *et al*., 1994). For example, PUT and CAD are known to enhance HIS toxicity (Lehane and Olley, 2000). HIS is hazardous for human health at a concentration higher than 100 ppm in the main diet. It has been reported that 100 to 800 ppm of TYR in food is toxic and 6 mg of TYR caused hypertensive symptoms during standard monoamine oxidase inhibitor treatments (Ten-blink *et al*., 1990; Rauscher-Gabernig *et al*., 2009). Over 1000 ppm of total BAS in food is considered hazardous to health (Santos, 1996). The BAS levels in Thai fermented SS and SBP studied were mostly lower than the values considered hazardous to human health. Although SS and SBP are used as condiments or flavoring agents which are not the main diet, high concentration of these amines in the products may reflect inferior manufacturing practices (Tasić *et al*., 2012).

Conclusion

We determined BAS contents of Thai commercial SS and SBP products and showed that types and levels of BAS varied depending on brand and formula. PUT

and CAD were the most prevailing BAS in all Thai SS; while PUT, CAD and SPD were the most BAS found in all Thai SBP products. However, the BAS levels in Thai SS and SBP studied were mostly in the safety zone for consumers.

Acknowledgement

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