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Analytical Methods

Determination of biogenic amines in selected Malaysian food

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ABSTRACT

The biogenic amines tryptamine (TRP), putrescine (PUT), histamine (HIS), tyramine (TYR) and spermidine (SPD) were determined in 62 selected food items commonly consumed in Malaysia. This include the local appetisers "budu" and "cincalok", canned fish, salt-cured fish, meat products, fruit juice, canned vegetables/fruits and soy bean products. After the aqueous extraction, the samples were derivatised with dansyl chloride before analysing using reversed phase HPLC with UV detection. Mean levels of TRP, PUT, HIS, TYR and SPD in eight budu samples were 82.7, 38.1, 187.7, 174.7 and 5.1 mg kg^{-1}, respectively. The main biogenic amines found in cincalok were PUT, HIS and TYR where the mean values were 330.7, 126.1 and 448.8 mg kg^{-1}, respectively. With the exception of "pekasam" and "belacan", significantly lower levels of biogenic amines were found in canned fish and salt-cured fish samples. Non detectable or low levels of biogenic amines were found in meat, fruit juice and canned vegetables/fruit samples.

1. Introduction

Biogenic amines are low molecular weight organic bases of aliphatic, aromatic or heterocyclic structure that are synthesized and degraded during the cellular metabolism activities in microorganisms, plants and animals (Lapa-Guimarães & Pickova, 2004; Silla Santos, 1996; Tassoni, Germaná, & Bagni, 2004). Putrescine, spermidine and spermine occur universally in animals and plants, while putrescine and spermidine are also found in most bacteria (Shalaby, 1996). These amines are endogenous and indispensable components of living cells and are important in the cell proliferation and differentiation, regulation of nucleic acid function, protein synthesis, brain development, nerve growth and regeneration (Eliassen, Reistad, Risøen, & Rønning, 2002; Kalač & Krausová, 2005; Silla Santos, 1996; Tassoni et al., 2004). In plants, they have also been associated with pH and thermic or osmotic stress responses, cell division, flowering, and may function as allelochemical compounds and as components of the chemical and physical defenses against herbivores and pathogens (Bouchereau, Guénot, & Larher, 2000).

Biogenic amines can also be found in a variety of foods, beverages and fermented foods especially in protein-rich foods e.g., fish and fish products, meat and meat products, eggs, cheeses, fermented vegetables, fruits, nuts, chocolate, soy bean products, beers and wines (Kalač, Švecová, & Pelikánová, 2002; Kvasnička & Voldřich, 2006; Shalaby, 1996; Silla Santos, 1996). Biogenic amines in foods and beverages are formed by microbial decarboxylation of the corresponding amino acids (Awan, Fleet, & Thomas, 2008; Lapa-Guimarães & Pickova, 2004; Silla Santos, 1996). It was also found that biogenic amines are not significantly reduced by high temperature treatment (Shalaby, 1996; Silla Santos, 1996).

The determination of biogenic amines in foods is of great interest not only due to their possible toxicity, but also can be used as indicators for quality of freshness or spoilage of foods (Awan et al., 2008; Silla Santos, 1996). Biogenic amines, especially histamine, putrescine and cadaverine have been suggested as indicators of spoilage of some foods, such as fresh fish, meat and vegetables (Riebroy, Benjakul, Visessanguan, Kijrongrojana, & Tanaka, 2004). The amount and type of biogenic amines formed is strongly influenced by the food composition, microbial flora and by other parameters which allow bacterial growth during food processing and storage (e.g., food treatment prior to storage, food additives, temperature, moisture, ripening and packaging) (Carelli, Centonze, Palermo, Quinto, & Rotunno, 2007; Draisci et al., 1998).

Low levels of biogenic amines in food are not considered a serious risk. However, when consumed in excessive amounts, they may cause distinctive pharmacological, physiological and toxic effects. It is worthy to mention that there is also evidence of linkaging elevated biogenic amine levels and cancer (Tassoni et al., 2004). It had been reported that high polyamine concentrations in breast and colon cancer cells (Paproski, Roy, & Lucy, 2002).

Several analytical methods for the determination of biogenic amines in foods have been described. These include thin layer chromatography (Lapa-Guimarães & Pickova, 2004), biosensors (Carelli et al., 2007; Draisci et al., 1998; Keow et al., 2007), capillary

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electrophoresis (Kvasnička & Voldřich, 2006; Paproski et al., 2002) and reversed phase high performance liquid chromatography (HPLC) (Kalač et al., 2002; Lange, Thomas, & Wittmann, 2002; Moret, Smela, Populin, & Conte, 2005; Oguri, Enami, & Soga, 2007; Tassoni et al., 2004). Positive confirmation using mass spectrometry either after HPLC (Gosetti, Mazzucco, Gianotti, Polati, & Gennaro, 2007; Kirschbaum, Rebscher, & Brückner, 2000) or gas chromatographic separation (Awan et al., 2008) have also been reported. Of these HPLC is the most popular and frequently reported for the separation and quantification of biogenic amines.

Since many biogenic amines occurring in food show neither satisfactory absorption, nor exhibit significant fluorescence properties, chemical derivatization is usually performed to increase the sensitivity. Many derivatisation reagents have been described. Of these, *o*-phthalaldehyde (OPA) and dansyl chloride are the most widely used. The use of OPA results in the formation of rapid and sensitive derivatives with primary amines. However, dansyl chloride forms derivatives not only with primary but also secondary amines. Furthermore, the products are more stable than those formed by using OPA (Anli, Vural, Yilmaz, & Vural, 2004; Lapa-Guimarães & Pickova, 2004).

Fermented food is widely consumed all over the world. Of particular interest to us are the indigenous fermented food that are prepared from fish and shrimps (known locally as "budu" and "cincalok") that form a popular appetizer that is consumed in countries of South East Asia. These items are normally consumed with fresh local herbs and eaten with rice. No previous reports on the analysis of biogenic amines in these items have been reported. The five analytes studied (TRP, PUT, HIS, TYR and SPD) are among the most prevalent biogenic amines found in fermented foods. Besides that, other commonly consumed food items such as salt-cured fish, meat products, fruit juice, canned vegetables/fruits and soy bean products are also analysed. The biogenic amines analysed are shown in Table 1.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and solvents used were of analytical and chromatographic grade, respectively. Spermidine trihydrochloride (SPD), putrescine dihydrochloride (PUT), histamine dihydrochloride (HIS) and tryptamine hydrochloride (TRP) were purchased from Sigma–Aldrich. Tyramine hydrochloride (TYR) and dansyl chloride were obtained from Fluka. Acetone and sodium hydroxide were purchased from Systerm while glutamic acid monosodium monohydrate was from Acros Organics. Sodium hydrogen carbonate was purchased from BDH Chemicals. Hydrochloric acid (HCl) was obtained from Lab Scan and trichloroacetic acid (TCA) was from R & M Chemicals. HPLC grade acetonitrile was obtained from Fisher Scientific. Milli-Q water produced from a Nanopure Diamond, Barnstead unit was used throughout.

2.2. Preparation of standard solutions

Stock solution (1000 mg L^{-1}) of a mixture of TRP (61.4 mg), PUT (91.5 mg), HIS (82.8 mg), TYR (63.2 mg) and SPD (87.7 mg) was prepared in 0.1 M HCl in a volumetric flask (50 mL). The solution was stored in the dark at 4 °C. Standard solutions were prepared by diluting from this stock solution and used to obtain the calibration curves.

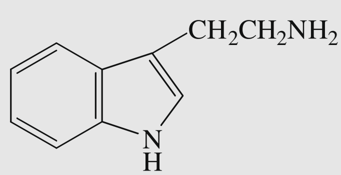
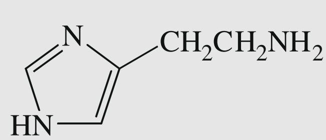
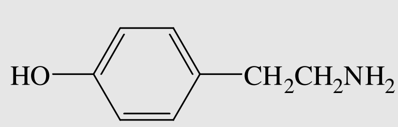
2.3. Food samples

Food samples were purchased from supermarkets, wet and night markets from the states of Penang, Kelantan and Melaka, Malaysia. Sixty-two food samples were analysed and were categorised as canned fish (7), salt-cured fish (13), fermented fish sauce (budu) (8) and shrimp sauce (cincalok) (10), meats (5), canned vegetables/fruits (6), juices (8) and soy bean products (5). Frozen samples were thawed prior to analysis.

2.4. Preparation of food samples

Different preparation procedures were used according to the type of sample. 5% TCA was used for the extraction of fish and meat products while 0.1 M HCl was used for the extraction of the other samples. Liquid samples were diluted ten times with the respective acid. All solid samples were homogenised prior to the extraction using a blender. Five grams of the resulting slurry was extracted twice with 25 mL of the respective acid and homogenised using a vortex mixer for 3 min. The homogenate was centrifuged

Table 1
Biogenic amines studied (Gugliucci, 2004; Kvasnička & Voldřich, 2006)

Name	Abbreviation	Structure	Molecular weight	p*K*
Tryptamine	TYP		160.2	p*K* = 10.2
Putrescine	PUT	$H_2N(CH_2)_5NH_2$	88.2	pK^1 = 10.8, pK^2 = 9.4
Histamine	HIS		111.1	pK_1 = 9.8, pK_2 = 6.0
Tyramine	TYR		137.2	p*K* = 9.6
Spermidine	SPD	$H_2N(CH_2)_3NH(CH_2)_4NH_2$	145.3	pK_1 = 9.5, pK_2 = 10.8 pK_3 = 11.6

(3000 rpm) for 15 min. For fish and meat samples, the homogenates were stored at 4 °C to allow the precipitation of fats and proteins. The supernatant were combined and filtered through a Whatman no. 1 filter paper. One milli liter of the extract was used for the analysis after derivatization with dansyl chloride.

2.5. Derivatization procedure

One micro liter of sample was mixed with a saturated solution of $NaHCO_3$ (200 µL), 2 M NaOH (20 µL) and 2 mL of dansyl chloride (10 mg of dansyl chloride in 1 mL acetone). The mixture was heated in a water bath (70 °C) for 10 min. After the reaction, the unbound dansyl chloride was removed by adding 1 mL of glutamic acid (50 mg of glutamic acid in 1 mL water). After 1 hour, the mixture was adjusted to 5 mL with acetonitrile. The solution was filtered through a 0.45 µm membrane filter and injected onto the chromatographic column.

2.6. Instrumentation and chromatographic conditions

Quantitative analysis of biogenic amines were carried out using a HPLC unit that consisted of a pump (Jasco PU-1580), quarternary gradient (Jasco LG-1580-04), UV/VIS detector (Jasco UV-1570), injector and a 20 µL sample loop (Jasco model 7725). Separation was achieved using a Waters Spherisorb 5 µm ODS2 column (250 × 4.5 mm). The mobile phase was acetonitrile: water (67:33, v/v) and the flow rate was 1.2 mL min^{-1}. The mixture was filtered using membrane filter and degassed in an ultrasonic bath for 15 min prior to use. The detector was set at 254 nm.

3. Results and discussion

3.1. Analytical characteristics of the method

Biogenic amines were analysed according to the method as described by Moret et al. (2005). The analytical method was validated by determining the linear range, limits of detection (LOD) and quantification (LOQ), precision and recovery. Results are summarised in Table 2. Linearity of the calibration curves was established by injecting five concentrations of standard mixtures (0.1–250 mg kg^{-1}). Good linearity (R^2: 0.9979–0.9999) was obtained between peak area and analyte concentration.

The LOD was determined from the minimum concentration of the amine required to give a signal to noise ratio of three while the LOQ were determined with a signal to noise ratio of 10. The sensitivity of the method, as reflected by the LOD and LOQ values, is comparable to those reported in the literature (Gosetti et al., 2007; Shakila, Vasundhara, & Kumudavally, 2001).

The repeatability and reproducibility of the method was assessed by injecting five times each of the five standard mixtures on the same day (intraday) and over seven days (interday), respectively. Good reproducibility of both the peak area (RSD ⩽ 3.09%) and retention times (RSD ⩽ 0.71%) were found (Table 2).

Recovery studies were carried out by spiking three concentrations (1, 5 and 10 mg kg^{-1}) of a mixture of amine standard to fish, meat, juice, soy bean and vegetable samples. The mixture was derivatised and injected into the HPLC column. Satisfactory recovery for all amines was obtained (90.9–106.3%, Table 3).

3.2. Analysis of food samples

In the analysis of food samples, peak identification was based on the comparison between the retention times of standard compounds and was confirmed by spiking standards to the samples. Quantification was based on the external standard method using calibration curves fitted by linear regression analysis. Under the stated experimental conditions, baseline separation of the five biogenic amines was achieved in less than 30 min. No interfering peaks appeared at the retention times of the analytes (Fig. 1). The method was applied for the determination of biogenic amines in 62 different food samples. The food samples tested and the levels of the biogenic amines found are shown in Table 4.

Table 2
Analytical characteristics of the HPLC method

Biogenic amine	Linear range (mg L^{-1})	R^2	LOD (µg L^{-1})	LOQ (µg L^{-1})	Intraday (% RSD) (n = 3)		Intraday (% RSD) (n = 3)	
					t_R	Area	t_R	Area
TYP	0.1–250	0.9989	5.36	17.88	0.71	0.42	0.34	2.38
PUT	0.1–100	0.9999	4.43	14.76	0.49	0.86	0.39	2.47
HIS	0.1–130	0.9993	5.11	17.04	0.34	0.18	0.47	3.09
TYR	0.1–200	0.9995	7.34	24.45	0.14	2.07	0.48	2.72
SPD	0.1–130	0.9979	6.96	23.19	0.09	2.12	0.44	2.69

R^2, square of regression coefficient; tR, retention time; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation.

Table 3
Recoveries and RSD of biogenic amines when spiked to different food samples

Biogenic amine	% Recovery														
	1 mg L^{-1}					5 mg L^{-1}					10 mg L^{-1}				
	Fish	Meat	Juice	Soy bean	Vegetable	Fish	Meat	Juice	Soy bean	Vegetable	Fish	Meat	Juice	Soy bean	Vegetable
TYP	96.9	95.2	101.3	96.2	96.8	100.0	98.2	101.9	97.4	96.7	103.7	100.6	94.7	97.4	94.7
	(0.6)	(1.2)	(3.8)	(4.7)	(4.7)	(2.7)	(1.2)	(0.3)	(4.1)	(1.6)	(0.6)	(0.6)	(1.9)	(1.4)	(0.8)
PUT	97.3	96.7	95.1	96.7	98.7	104.9	103.7	103.6	103.7	106.3	103.9	92.3	99.2	99.4	99.2
	(0.9)	(1.0)	(1.6)	(1.9)	(4.3)	(1.4)	(1.2)	(0.6)	(4.7)	(0.2)	(2.5)	(0.7)	(0.8)	(0.4)	(0.8)
HIS	94.0	96.1	95.2	100.8	98.5	99.6	97.5	105.4	97.3	99.6	104.1	102.1	102.4	99.1	102.4
	(4.7)	(2.1)	(3.7)	(2.4)	(2.9)	(1.0)	(1.6)	(0.1)	(4.2)	(3.1)	(1.2)	(1.1)	(2.0)	(1.1)	(2.0)
TYR	98.8	96.1	98.6	95.4	94.3	101.7	103.2	101.8	102.6	102.4	98.4	95.6	102.9	102.7	102.9
	(3.1)	(2.1)	(0.4)	(4.9)	(4.5)	(3.1)	(2.3)	(5.2)	(2.8)	(0.7)	(0.8)	(1.9)	(1.7)	(0.1)	(2.2)
SPD	90.9	102.1	99.5	95.4	93.9	99.9	98.3	93.4	98.9	103.7	96.6	104.9	97.1	96.5	97.1
	(5.3)	(3.1)	(4.9)	(4.9)	(1.2)	(0.2)	(1.9)	(1.5)	(4.9)	(1.2)	(0.7)	(2.1)	(2.2)	(1.9)	(2.2)

Values in brackets denote relative standard deviation (RSD) (n = 3).

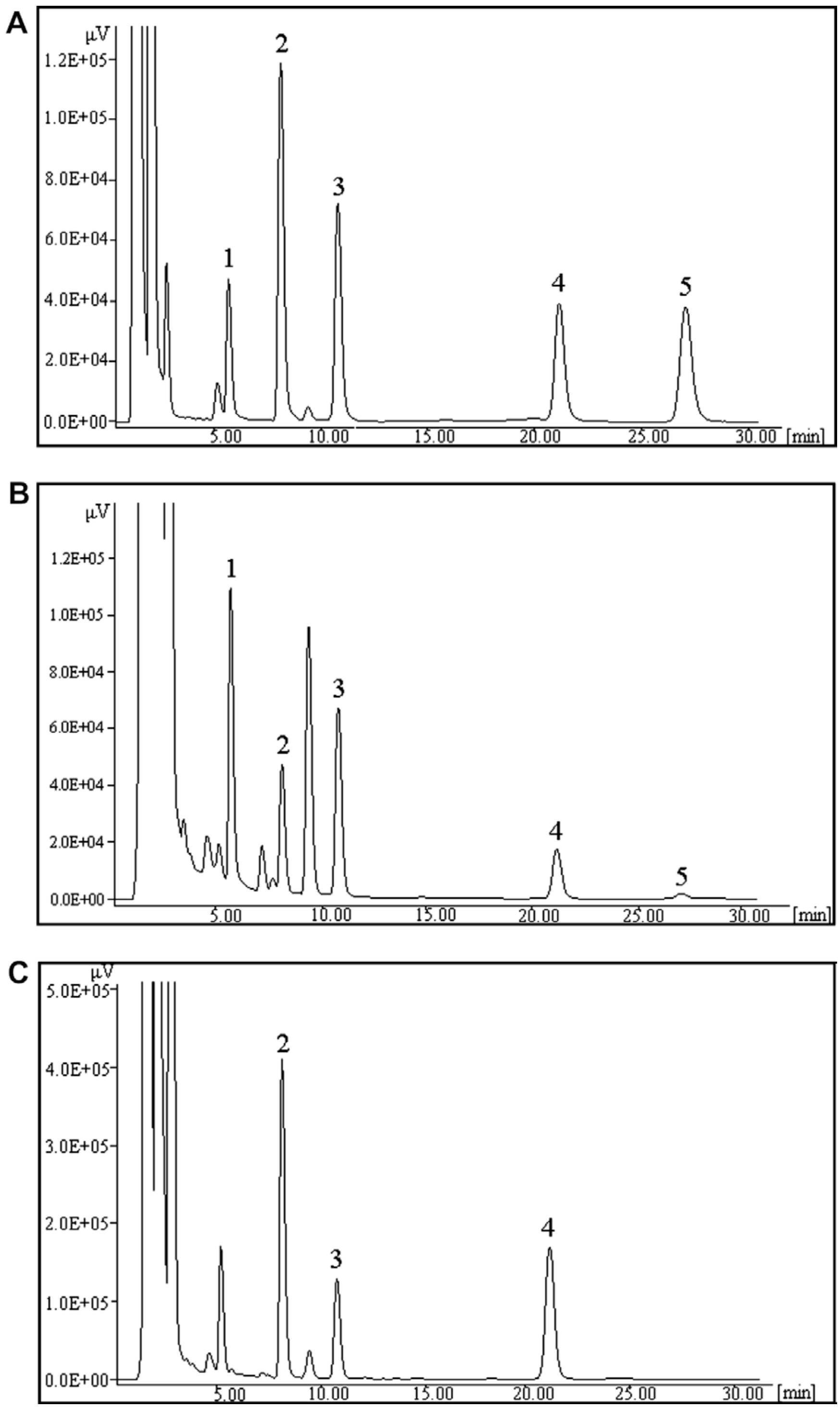


Fig. 1. Typical HPLC chromatograms of (A) biogenic amines standard solution (5 mg L^{-1} each), (B) budu (sample no. 6), (C) cincalok (sample no. 9), (D) canned fish (sample no. 19), and (E) salt-cured fish (sample no. 26) extracts. Peak identity: TRP (1), PUT (2), HIS (3), TYR (4), and SPD (5).

"Budu" is a popular appetiser especially among the population of the East Coast states of Malaysia. It is traditionally made by mixing anchovies and salt (2–6:1 w/w) and was allowed to ferment for 140–200 days. The fermentation process is carried out by halophilic microorganisms, which partially belong to the native microflora of fish (Kirschbaum et al., 2000). The budu samples investigated showed mean levels of TRP (82.7 mg kg^{-1}), PUT (38.1 mg kg^{-1}), HIS (187.7 mg kg^{-1}) and TYR (174.7 mg kg^{-1}) as the major source of biogenic amines. PUT (330 and 101.4 mg kg^{-1}), HIS (126.1 and 27.9 mg kg^{-1}) and TYR (448.9 and 63.2 mg kg^{-1}) are the major biogenic amines found in "cincalok" and salt-cured fish, respectively.

TYR is usually the major amine found in fermented products (Riebroy et al., 2004). Good Manufacturing Practice stipulates that 100–800 mg kg^{-1} for TYR is regarded as acceptable, at 1080 mg kg^{-1} is toxic and over 100 mg kg^{-1} may cause migraine (Shalaby, 1996). It was reported that TYR levels can change on storage even at refrigerator temperatures, and it is possible that foods thought

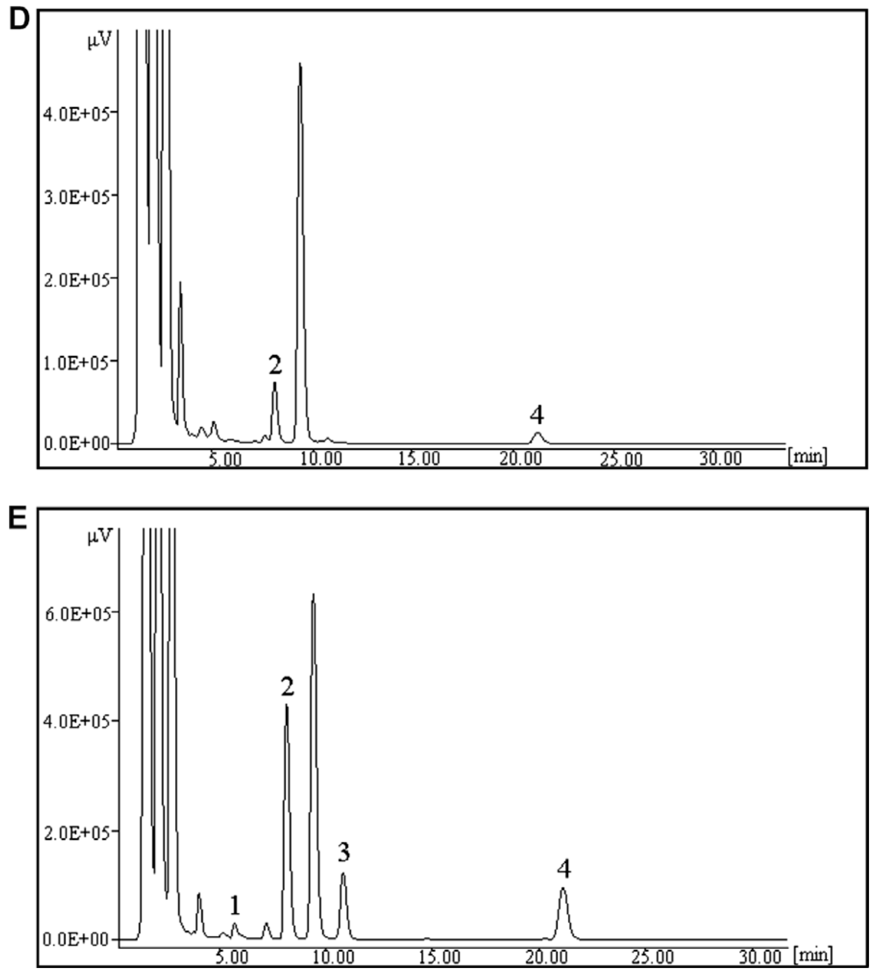


Fig. 1 (*continued*)

to be TYR free might be a cause of concern if an unusually long time elapses between purchase and consumption (Mower & Bhagavan, 1989). The authors also observed that TYR content of shrimp sauce in ethnic foods found in Hawaii contained relatively high levels of TYR (245.5 mg kg^{-1}).

Reports by Kirschbaum et al. (2000) also found that the fish sauce tested showed high amounts of TYR, PUT, TRP and HIS (all exceeding 100 mg kg^{-1}). The present results agree to the findings of Veciana-Nogués, Vidal-Carou, and Mariné-Font (1989) that HIS and TYR were higher in semi-preserved anchovies than in canned fish products. Stratton, Hutkins, and Taylor (1991) also reported that fermented fish products frequently contained high amounts of HIS.

The higher amine contents in anchovy products was explained by the poor quality raw material and/or by formation during the maturation process involving microorganisms, long processing time, storage under improper conditions, inadequate handling and the absence of preservatives (Silla Santos, 1996; Veciana-Nogués et al., 1989). Furthermore, the finished product is stabilised without heat treatment, and therefore, the amine-forming microbial activity may continue, especially if it is stored under favourable conditions (Santos-Buelga, Marine-Font, & Rivas-Gonzalo, 1986). The levels of free amino acids usually increase in fishery products during storage due to the action of endogenous and exogenous proteases through proteolysis process (Makarios-Laham & Lee, 1993). It is thought that proteolysis might provide the nutrient for spoilage microorganisms, leading to the promoted growth of those microorganisms (Riebroy et al., 2004).

According to the Compliance Policy Guideline No. 540.525 (Federal Register, 1995), good quality fish should contain less than 10 mg kg^{-1} of HIS, 30 mg kg^{-1} indicates significant deterioration, and 50 mg kg^{-1} is considered to be conclusive evidence of decomposition (Oguri et al., 2007). The FDA has established a 'hazard action level' of 500 mg kg^{-1} for HIS (Keow et al., 2007). Based on the assessment of poisoning cases, the guidance levels suggested for HIS content of fish are <50 mg kg^{-1} for safe consumption; 50–200 mg kg^{-1} possibly toxic, 200–1000 mg kg^{-1} probably toxic and >1000 mg kg^{-1} toxic and unsafe for human consumption (Proestos, Loukatos, & Komaitis, 2008). An intake of more than 40 mg biogenic amines per meal has been considered potentially toxic (Shalaby, 1996). However, not all amine are of equal toxicity, consequently the levels of HIS and TYR are of concern.

Different levels of HIS are regulated in different countries, e.g., 50 mg kg^{-1} is proposed by the US Food and Drug Administration (FDA). Higher levels are recommended by the European Community, South Africa and Italy (100 mg kg^{-1}), and Australia and Germany (200 mg kg^{-1}) (Auerswald, Morren, & Lopata, 2006; Carelli et al., 2007; Lange et al., 2002; Veciana-Nogués, Mariné-Font, & Vidal-Carou 1997).

PUT is the most abundant biogenic found in cincalok. Low levels of this amine has been reported as natural amine in animal and vegetable tissues, which are formed from the terminal products of the post mortem decomposition of organic matter (Shalaby, 1996; Silla Santos, 1996). While PUT has no adverse health effects, but it can potentiate the toxic effects of TYR and HIS by competing for the detoxifying enzymes and may react with nitrite to form

Table 4
Levels of biogenic amines found in tested samples ($n = 3$)

Sample no.	Sample type	Concentration, mg kg^{-1}				
		TRP	PUT	HIS	TYR	SPD
Fish sauce ("budu")						
1	Budu 1	317.6	28.4	372.9	852.6	3.0
2	Budu 2	ND	64.9	290.6	145.4	4.8
3	Budu 3	ND	32.5	180.9	62.4	5.0
4	Budu 4	ND	41.9	160.6	62.1	6.6
5	Budu 5	34.4	33.2	154.4	58.0	7.0
6	Budu 6	246.1	34.4	124.4	65.4	4.7
7	Budu 7	34.8	30.9	118.6	82.2	5.0
8	Budu 8	28.9	38.9	99.0	69.6	6.6
	$\bar{x}$	82.7	38.1	187.7	174.7	5.1
Shrimp sauce ("cincalok")						
9	Cincalok 1	ND	473.3	257.8	681.2	ND
10	Cincalok 2	ND	569.3	44.8	677.1	ND
11	Cincalok 3	ND	803.4	152.7	619.9	ND
12	Cincalok 4	ND	464.0	143.1	634.7	ND
13	Cincalok 5	ND	269.5	155.0	544.2	ND
14	Cincalok 6	ND	378.8	101.7	377.3	ND
15	Cincalok 7	25.3	65.4	178.6	254.3	2.2
16	Cincalok 8	ND	176.6	191.7	271.6	ND
17	Cincalok 9	ND	96.3	28.4	272.2	ND
18	Cincalok 10	ND	10.6	6.8	156.1	ND
	$\bar{x}$		330.7	126.1	448.9	
Canned fish						
19	Salty mackerel in oil	ND	56.2	ND	54.4	ND
20	Pink salmon in oil	65.5	5.5	ND	ND	6.1
21	Tuna flakes in oil	6.0	3.1	18.0	1.8	1.4
22	Tuna chunks in water	13.4	2.4	2.1	23.3	1.2
23	Anchovy in chilli sauce	8.4	0.3	2.0	2.1	1.6
24	Sardines in tomato sauce	15.2	ND	ND	ND	2.4
25	Pink salmon in water	19.3	6.2	ND	1.2	3.6
	$\bar{x}$	18.2	12.3	3.2	11.8	
Salt-cured fish						
26	Pekasam	64.9	416.2	195.0	369.4	ND
27	Shrimp paste ("Belacan")	ND	658.1	31.2	242.5	ND
28	Anchovy	ND	5.7	3.2	30.5	61.8
29	Dried shrimp	ND	8.4	2.1	2.4	ND
30	Parang	ND	84.1	ND	77.0	2.4
31	Kembong	ND	4.2	111.8	ND	3.6
32	Gelama	13.4	101.2	ND	67.0	2.4
33	Dried gelama	ND	13.7	ND	7.5	0.8
34	Merah	ND	8.3	ND	1.5	1.0
35	Duri	4.4	4.0	2.0	5.7	1.5
36	Kurau	ND	5.6	1.1	5.8	0.7
37	Talang	ND	4.9	13.1	5.7	0.8
38	Sepat	18.1	4.4	3.5	5.9	3.7
	$\bar{x}$		101.4	27.9	63.2	
Meat						
39	Beef sausage	ND	ND	ND	ND	ND
40	Beef salami	3.0	ND	3.9	1.0	ND
41	Frozen minced beef	ND	1.5	1.8	1.2	1.4
42	Beef green peppercorn	ND	ND	ND	ND	0.8
43	Beef burger	1.3	ND	ND	8.5	ND
Fruit juice						
44	Mango	14.1	ND	11.5	ND	ND
45	Orange	14.0	6.4	14.9	1.2	ND
46	Blackcurrant	ND	1.0	1.5	ND	2.1
47	Apple	ND	5.1	ND	ND	2.0
48	Pineapple	ND	ND	ND	ND	2.7
49	Red grape	ND	ND	7.4	ND	1.5
50	Lychee	ND	ND	9.4	ND	ND
51	Water chestnut	ND	ND	11.5	ND	ND
Canned vegetables/fruits						
52	Prune in brine	6.2	1.2	ND	ND	ND
53	Sour ginger	ND	6.5	ND	ND	2.0
54	Pickled leeks	ND	3.0	ND	ND	ND
55	Salted plum	12.0	ND	ND	ND	ND
56	Tomato puree	ND	1.7	ND	ND	2.4
57	Tomato ketchup	ND	3.6	ND	ND	3.4
Soy bean products						
58	Tempe	15.6	116.9	4.1	4.3	11.6
59	Soy bean sauce	ND	1.0	9.6	1.0	ND
60	Salty soy sauce	ND	ND	2.0	ND	ND
61	Taucu (salty bean)	ND	59.0	0.8	ND	ND
62	Soya bean milk	20.2	ND	17.5	1.7	1.3

ND-not detected.

heterocyclic carcinogenic nitrosamines (Silla Santos, 1996). PUT has also been proposed as spoilage indices in fish (Riebroy et al., 2004). Small amounts of SPD was found in most of the fish samples, mainly due to the fact that this amine plays an important role in live fish metabolism and cellular growth (Shalaby, 1996; Silla Santos, 1996). Bardócz (1995) reported that all types of foods, whether they originate from plant or animal sources, contained PUT and SPD, while fish has higher levels of PUT than SPD.

Generally, the canned fish analysed contained low levels of biogenic amines, except for salty mackerel in oil (sample no. 19) and pink salmon in oil (sample no. 20). Shakila et al. (2001) found significantly lower levels of these amines, while Eliassen et al. (2002) found relatively high levels of these amines in canned fish. Salt-cured fish also contained low level of biogenic amines, except for pekasam (sample no. 26), belacan (sample no. 27), parang (sample no. 30), and gelama (sample no. 32). HIS levels in pekasam and kembong were both above 100 mg kg^{-1}. Similar results were reported by Shakila et al. (2001), who found relatively high levels of biogenic amines in salt-dried fish.

Relatively low levels of biogenic amines were found in meat, fruit juice and canned vegetable/fruit samples. Low levels of biogenic amines in sausage and salami were reported by Bardócz (1993) and Lange et al. (2002), respectively. Hernandez-Jover, Izquierdo-Pulido, Veciana-Nogues, Marine-Font, and Vidal-Carou (1997) emphasized that SPD is always detected in meat and meat products and can occur naturally in fresh beef, and their formation is not due to food spoilage or fermentation processes, while Kalač and Krausová (2005) reported that SPD content in meat rarely exceed 10 mg kg^{-1}.

Moret et al. (2005) showed that vegetables generally contained low levels in biogenic amines (0.1–9.6 mg kg^{-1}) while Kalač et al. (2002) found relatively high levels of the amines in vegetables (0.8–52.5 mg kg^{-1}). The polyamines PUT and SPM are practically ubiquitous in all vegetables at a few mg/100 g of fresh weight and TYR is less widespread in vegetables (Kalač et al., 2002; Moret et al., 2005). They are implicated in a number of physiological processes, such as cell division regulation, plant growth, flowering, fruit development, response to stress and senescence (Bouchereau et al., 2000). Moreover, although PUT, SPD and other biogenic amines are generated in low quantities in most canned vegetables/fruits, they are not the primary metabolic products produced by the fermenting organisms (Stratton et al., 1991).

Substantial quantities of PUT may be characteristic of citrus fruits, such as orange as well as orange and grapefruits juices (Bardócz, 1993; Eliassen et al., 2002). It was in fact reported that orange fruits and juices contained more PUT than other fruits and vegetables (Tassoni et al., 2004). It had been reported that PUT was the predominant amine in most fruit juice samples including raspberries, lemons, grapefruits, mandarins, strawberries, currents and grapes (Shalaby, 1996). Moret et al. (2005) noted that free biogenic amines in fruits and vegetables shape the typical and characteristic taste of mature foods and are precursors of certain aroma compounds.

With the exception of tempe (sample no. 58) and taucu (sample no. 61), relatively low levels of biogenic amines are found in the soy bean products tested. Studies by Mower and Bhagavan (1989) showed higher level of TYR (450 mg kg^{-1}) in salted black beans. Since several varieties of molds, yeasts and lactic acid bacteria are involved in the fermentation processes of such products and the raw material (soy bean) contains considerable amounts of protein, the formation of various amines might be expected during the fermentation (Shalaby, 1996). Studies have shown that biogenic amines in fermented soy bean products are most likely formed by the lactic microflora that is active during fermentation (Kirschbaum et al., 2000; Stratton et al., 1991). TYR and HIS have been found at various levels in such products (Stratton et al., 1991). The variability of biogenic amines levels in the commercial fermented soy bean products samples had been attributed to the variations in manufacturing processes; variability in the ratio of soy bean in the raw material, microbial composition, conditions and duration of fermentation (Shalaby, 1996).

4. Conclusion

This study indicate that the presence of biogenic amines in canned vegetable/fruit, juice and soy bean products do not present a health risk for consumers due to their relatively low levels. HIS and TYR were found at 6.8–372.9 and 58.0–852.6 mg kg^{-1}, respectively, in budu and cincalok. However, no samples in this study contained HIS that exceed the recommended action level of 500 mg kg^{-1}.

To the best of our knowledge, no cases of poisoning due to excessive intake of food items such as budu or cincalok have been reported in Malaysia. Although the levels of some of the biogenic amines are relatively high in some of these food items, the fact that generally they are not consumed on a regular basis probably accounts for the lack of reported poisoning cases. However, excessive intake of these food items on a regular basis is not recommended.

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