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Biogenic amines analysis in shrimp pastes belacan obtained from the Northern States of Peninsular Malaysia

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Abstract

Shrimp paste or belacan production is still largely a cottage industry that generated additional income to many homestay operators or family that involve in fisheries. Belacan production was inconsistent depending on the availability of *Acetes* shrimp. Market shelf life and quality between products varies as small production volume caters for certain places. A study was initiated to determine the biogenic amines content and several physico-chemical parameters in belacan obtained from the Northern region, Malaysia. Biogenic amines in sample was extracted using 0.1 M acid hydrochloric (HCl), derivatized with dansyl chloride, separated using ODS Hypersil LC-18 column (250 x 4.6 mm, 5 µm particle size) and elucidated for chromatographic analysis using HPLC HITACHI L4250 UV-Vis. Good linearity with square of regression coefficient (r^2) ranging from 0.997-0.999 were obtained for putrescine, cadaverine, histamine and tyramine with LOD ranging from 0.13 mg L^{-1} for tyramine to 0.57 mg L^{-1} for cadaverine and LOQ of 0.45 mg L^{-1} for tyramine to 1.89 mg L^{-1} for cadaverine. About 47% of samples contain histamine below 50 mg kg^{-1} (ppm) safe limit. The highest histamine level was 143 mg kg^{-1} (mean, 57.6 mg kg^{-1}). The average of putrescine, cadaverine and tyramine were 137, 50.1 and 8.64 mg kg^{-1} with the highest recorded value of 314, 134 and 20 mg kg^{-1}, respectively (n=15). The salt percentage was higher than 17% whereas the moisture was below 40% in 73% of samples. These findings indicate the need to monitor the histamine content in belacan products for quality and safety assurance.

Introduction

Biogenic amines are group of low molecular weight organic bases that are synthesized and degraded as part of cellular metabolism activities by microorganisms, plant and animals (Silla Santos, 1996). Biogenic amines have been implicated in a variety of cell functions involving cell growth and differentiation and receptor function (Baron and Stasolla, 2008). The relevant importance of biogenic amines is their potential toxicity associated with high accumulation and consumption of histamine in fish and fisheries products (Hungerford, 2010). Histamine has been used as indicator of food freshness whereas high level of putrescine and cadaverine indicate deterioration in food products associated with undesired microbial activities or their defective manufacture (Lehane and Olley, 2000).

Biogenic amines are known to occur in a wide variety of fermented and non-fermented foods, such as fish, meat, dairy, fruits and vegetables, (Sillla Santos, 1996; Steinkraus, 1996). The determination of biogenic amines in fermented food products is critical in term of their potential health risks before consumption. Food-fermenting lactic acid bacteria which involve in fermentation process are generally considered to be non-toxic and non-pathogenic, however, certain species of lactic acid bacteria produce biogenic amines in food by microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes and ketones (Brink *et al.*, 1990; Maijala and Nurmi, 1995; Zolou *et al.*, 2003; Lopamudra *et al*., 2015).

Biogenic amines accumulation in fermented foods is a complex process, affected by many factors, and interaction between them. The cleanliness of raw ingredient has a strong influence on the growth of spoilage microbes. Many common contaminants and normal flora of aquatic life found in fermented products, to some extent, are biogenic amines-forming bacteria including some of the microbes involved in fermentation process. Thus, fermented foods normally contain a considerable amount of biogenic amines. Common perception that highly

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salted preserved food products are free from high biogenic amines accumulation are not entirely true as certain microbes are not totally killed or suppressed.

In Malaysia, as well as other Southeast Asian regions, shrimp paste production is not well standardized. The fermentation technique is still artisanal and carry out on small scale (Anna Rose and Pilapil, 2013). Transfer of knowledge about the production depends from families to families, and is passed based on experience. The quality of the same fermented products is not the same for one seller to another (Olympia, 1992; Putro, 1993). Thus, effort on studies regarding the quality and safety aspect of shrimp paste is still essential.

Fish sauce, fish and shrimp paste have been reported to contain high level of histamine with an average of 394, 263 and 382 mg kg^{-1} (ppm) respectively with 26% contained more than 500 ppm of histamine (Tsai *et al*., 2005). High putrescine and tyramine (658 and 242 mg kg^{-1}, respectively) with histamine of 31.2 mg kg^{-1} was reported in belacan (Saaid *et al*., 2009). Different profile of amines was reported in different types of products but information on local belacan is scarce due to the perception that highly salted (≥15% sodium chloride) dry formed fermented products are less risky than liquid form fermented sauce. Thus, the objective of this study is to determine the biogenic amines content and several physico-chemical parameters of local belacan from Northern region of Peninsular Malaysia.

Materials and Methods

Silver nitrate standard solution (0.1 M) and ammonium thiocyanate (NH_4SCN) standard solution (0.1 M) were obtained from R&M Chemicals (Malaysia), ferric ammonium sulphate ($FeNH_4(SO_4)_2.12H_2O$) from Merck (Germany). Putrescine dihydrochloride, histamine dihydrochloride, cadaverine dihydrochloride, tyramine hydrochloride and dansyl chloride were obtained from Sigma Aldrich (St. Louis, USA). For HPLC analysis, all chemicals and solvents used were of analytical and chromatographic grade, deionized water from Nanopure Diamond (Millipore, USA) and were used throughout the study.

pH measurement, sodium chloride (NaCl) and moisture percentage

10 g of sample (three replicates) was homogenized in 10 mL of distilled water (pH 7.0) and the pH was measured using pH meter (OHAUS Starter 3100, NJ, USA). Three independent values were obtained from each sample, means and standard deviations were calculated. Volhard back titration method was used for determination of sodium chloride (NaCl) in belacan (AOAC, 1995). Moisture in shrimp paste was determined by drying the sample to a constant mass at a specified temperature (105°C) in an oven. The results are expressed as ratio percentage of the mass of pore water to the mass of the solid material (AOAC, 2000).

Preparation of biogenic amines standard

Histamine (82.8 mg), putrescine (91.5 mg), cadaverine (85.7 mg) and tyramine (63.2 mg) were dissolved in 50 mM of 0.1 M hydrochloric acid (HCl) and used as the standard stock solution (1.0 mg L^{-1}). The method was validated in terms of analytical parameters such as linearity, precision (intra-day and inter-day repeatability), recovery (percentage), limit of detection (LOD) and limit of quantitation (LOQ) following the conventional protocols from the international guidelines (AOAC, 2002).

A series of each biogenic amines standard solutions (0.01, 0.1, 1, 10, 20, 50, 100 and 250 mg L^{-1}) were prepared to obtain the standard plots for histamine (HIS), cadaverine (CAD), putrescine (PUT) and tyramine (TYR). The linearity was established by injecting eight concentrations of standard mixtures (0.01, 0.1, 1, 10, 20, 50, 100, 250 mg L^{-1}) in three replicates. The calibration curves were plotted from the peak area and the corresponding concentration of biogenic amines. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 3 times and 10 times the standard deviation of the blank (0.1 M HCl) divided by the slope of the calibration curve for each analyte (HIS, CAD, PUT and TYR). Recoveries were obtained by spiking food matrices with four BA standards (PUT, CAD, HIS and TYR) at three different concentrations (1, 20 and 100 mg kg^{-1}). The recovery was calculated by comparing the amount of each biogenic amines detected in the spiked sample to the amount of each standard material added to the sample. The analysis was conducted in triplicate. The standards and stock solutions were kept refrigerated (4°C) in amber bottles.

Sampling method and sample extractions

Fifteen (n=15) belacan were purchased from a grocery stores, supermarkets and wet markets in Pulau Pinang, Kedah and Northern region of Perak in Peninsular Malaysia. A random sampling of belacan products found at local stores in the Northern Region of Peninsula Malaysia were categorized into the State of belacan produced. The weighed sample (0.5 g) was placed in sterile tube (15 mL) (Gibco, USA) and 0.1 M HCl solution was added to make up the 10

mL total volume. The mixture was homogenized by vortexed (VTX-3000L, Tokyo, Japan) for 3 minutes and centrifuged for 15 minutes at 4000 rpm (Model Kubota 2100, Selangor, Malaysia). The supernatant was collected by filtering through 0.45 µm pore size nylon syringe filter (Agilent, Germany). One mL of filtered solution (sample) was used to prepare a dansylated mixture for HPLC analysis.

Derivatization procedure

A mixture of sodium hydrogen carbonate ($NaHCO_3$)(200 µL), 2 M sodium hydroxide (NaOH) (20 µL) and 2 mL dansyl chloride (prepared by mixing 10 mg of dansyl chloride with 1 mL acetone) was added to 1 mL of standard and sample (filtered supernatant). The mixture was heated at 70°C for 10 minutes in a water bath before 1 mL of glutamic acid (50 mg in 1 mL water) was added to remove the unbound compounds. The mixture was further incubated for 1 hour before the dansylated solution was total up to 5 mL volume by addition of 780 µL acetonitrile (Saaid *et al.*, 2009). The final solution was filtered using 0.45 µm pore size nylon syringe filter (13 mm in diameter) (Agilent, Germany) before the analysis.

Instrumentation and chromatographic conditions

Analysis of biogenic amines was carried out using HITACHI high performance liquid chromatography (HPLC, Tokyo, Japan) consisting of L-6200 Intelligent Pump with L-4250 UV/Vis detector with manual injection syringe injector (20 µL). The separation was achieved using ODS Hypersil LC-18 HPLC column (25 cm x 4.6 mm, 5 µm size particle) (Thermo Scientific, USA). Data was analyzed using eDAQ PowerChrom 280 Ver. 2.7.2 data analysis. The isocratic elution program was used with acetonitrile: water: methanol (60:25:15) v/v as mobile phase at a flow rate of 1.0 mL min^{-1}. The mobile phase was filtered using membrane filter and degassed in an ultrasonic bath for 15 min prior to use. The detector was set at 254 nm.

Statistical analysis

One-way ANOVA based on Statistical Package Social Sciences v. 16.0 (SPSS Windows, USA) was used for statistical analysis for significance ($p<0.05$). The content of biogenic amines was determined as Mean±SD (three replicates, nine injections, n=9). Effect of NaCl%, pH and moisture% of belacan products on biogenic amines formation were analyzed using Pearson correlation analysis.

Results and Discussion

pH, sodium chloride and moisture content

Several states in the country such as Perlis, Kedah, Pulau Pinang and Melaka are well known for their belacan products. The details of local belacan products found in the market from the Northern States of Peninsular Malaysia are summarized in Table 1. The characteristics of belacan differ in term of appearance, colour, texture, taste and aroma depending on the manufacturing process and the type of raw material (shrimp species) and other ingredient added (Hajeb and Jinap, 2012). The pHs of belacan were neutral (6.8-7.6; mean, 7.2±0.27). The sodium chloride (NaCl) concentration varies between products ranging from 17.5-39.7% (mean, 24.9±5.97%). Results are shown in Table 2. About 27% of belacan samples contain moisture higher than 40% (range, 23.0-47.4; mean, 36.1±6.71). Under the Malaysian Food Regulation (1985), belacan should contain a minimum of 17% NaCl and less than 40% moisture. Pongsetkul *et al*. (2014) reported that the shrimp paste kapi from Thailand contained high salt ranging from 22.8-34.5%, moisture from 33.8-52.5% and pH of 7.01-8.4. Generally, the pH, moisture and salt percentage found in kapi did not differ much from belacan.

The statistical analysis using one-way ANOVA for pH, NaCl% and moisture% showed a significant different ($p<0.05$) between products at 95% confidence level regardless of its origin (State produced). About 40% of products contain high sodium chloride (NaCl) within range of 25-39.7%. Using Duncan homogeneous analysis, each group was significantly different with only one or two similarities within group.

Recovery, Linearity, LOD and LOQ

Biogenic amines were analyzed according to the method described by Moret *et al.* (2005). Good linearity with square of regression coefficient (r^2) ranging from 0.997-0.999 were obtained for putrescine, cadaverine, histamine and tyramine with LOD ranging from 0.13-0.57 mg L^{-1} and LOQ of 0.45-1.89 mg L^{-1}. Biogenic amines are baseline separated in less than 14 minutes. Typical chromatogram of biogenic amines standard is shown in Figure 1(a). Using a simple acidic extraction method (0.1 M HCl), good recoveries were obtained (70.4-114%) for putrescine, cadaverine, histamine and tyramine. Intra-day repeatability was within the acceptable range, ranging from 2.09-18.8 %RSD whereas inter-day repeatability ranges from 1.34-14.5 %RSD (Table 3).

Table 1. Details of belacan products studied

No.	Samples	Colour	State	Packing	Shape
1.	*Belacan* 1	Brown	Kuala Perlis	Plastic and oil paper wrapped	Round sliced
2.	*Belacan* 2	Reddish	Kuala Perlis	Plastic and printed paper wrapped	Round sliced
3.	*Belacan* 3	Light brown	Kuala Perlis	Plastic and oil paper wrapped	Round sliced
4.	*Belacan* 4	Dark brown	Balik Pulau, P. Pinang	Plastic sealed	Block
5.	*Belacan* 5	Dark brown	Seberang Perai Selatan, P. Pinang	Plastic and white glossy paper wrapped.	Block
6.	*Belacan* 6	Dark brown	Balik Pulau, P. Pinang	Plastic wrapped and paper box	Block
7.	*Belacan* 7	Dark brown	P. Pinang	Plastic and glossy paper wrapped	Block
8.	*Belacan* 8	Brown	Bukit Mertajam, P. Pinang	Plastic & box	Small cube
9.	*Belacan* 9	Light brown	Tanjung Dawai, Kedah	Double layer of plastic wrapped	Round sliced
10.	*Belacan* 10	Dark purplish	Kuala Kedah, Kedah	Plastic and oil paper wrapped	Round sliced
11.	*Belacan* 11	Brown	Kelemak, Melaka	Plastic	Round sliced
12	*Belacan* 12	Brown	Sri Damansara. Selangor	Plastic and box	Small cube
13.	*Belacan* 13	Brown	Balakong, Selangor	Plastic sealed	Round sliced
14.	*Belacan* 14	Light brown	No information	Plastic	Small cube
15.	*Belacan* 15	Dark brown	No information	Plastic & box	Small cube

Table 2. pH, sodium chloride (NaCl) and moisture content in belacan samples

Sample No.	pH	%NaCl	%Moisture
Belacan 1	7.5 ± 0.06^{a}	23.2 ± 0.36^{e}	46.5 ± 1.90^{j}
Belacan 2	7.5 ± 0.03^{a}	20.6 ± 0.75^{bc}	47.4 ± 0.68^{j}
Belacan 3	7.1 ±0.06^{c}	22.5 ± 0.14^{cde}	36.1 ± 0.10^{f}
Belacan 4	7.6 ± 0.03^{a}	21.5 ± 1.31^{cde}	33.6 ± 0.55^{de}
Belacan 5	6.9 ± 0.02^{b}	25.0 ± 0.91^{f}	34.0 ± 0.3^{ef}
Belacan 6	7.5 ± 0.08^{a}	22.6 ± 0.65^{cde}	42.2 ± 0.91^{h}
Belacan 7	7.3 ± 0.14^{d}	29.6 ± 1.46^{g}	35.5 ± 0.61^{ef}
Belacan 8	7.6 ± 0.08^{a}	35.3 ± 0.75^{i}	31.3 ± 0.35^{c}
Belacan 9	6.8 ± 0.03^{b}	19.3 ± 0.57^{b}	32.1 ± 0.06^{cd}
Belacan 10	7.1 ± 0.06^{c}	22.7 ± 0.79^{de}	43.8 ± 1.21^{h}
Belacan 11	7.3 ± 0.19^{d}	17.5 ± 0.39^{a}	38.6 ± 0.50^{g}
Belacan 12	7.3 ± 0.02^{a}	33.2 ± 0.18^{h}	34.0 ± 0.15^{de}
Belacan 13	7.3 ± 0.30^{d}	20.7 ± 0.96^{bcd}	26.8 ± 0.89^{b}
Belacan 14	6.8 ± 0.11^{b}	21.5 ± 0.17^{cde}	37.3 ± 2.41^{fg}
Belacan 15	6.9 ± 0.09^{b}	39.7 ± 2.97^{j}	23.0 ± 2.14^{a}
mean	7.2 ± 0.27	24.9 ± 5.97	36.1 ± 6.71

Values are Means ± S.D., values with different lowercase letters within the same column are significantly different ($p<0.05$) using one-way ANOVA.

Stability of the dansylated sample mixture was affected by many factors especially pH change with time (Eerola *et al.*, 1993; Romero *et al.*, 2000). In this study, variation in repeatability (%RSD) was found to be rather high (1.34-18.8 %RSD) using 0.1 M HCl but nevertheless, still within the acceptable limit (less than 20 %RSD). Saaid *et al.* (2009) showed much lower %RSD (0.6-5.3 %RSD) in intra-day repeatability (n=3) assay of fish sample using 5% trichloroacetic acid. In this study, the recovery using 5% trichloroacetic acid produced much lower recovery percentage (<70%) of biogenic amines standard compared when 0.1 M HCl acid was used for shrimp paste sample (97.4-114%) that was spiked with biogenic amines standard. One common problem that has been reported with the use of hydrochloric acid is the possible occurrence of turbidity (Innocente *et al*., 2007) that may have resulted in higher %RSD in the analysis.

Biogenic amines analysis of belacan

About 47% of samples (n=15) contain histamine

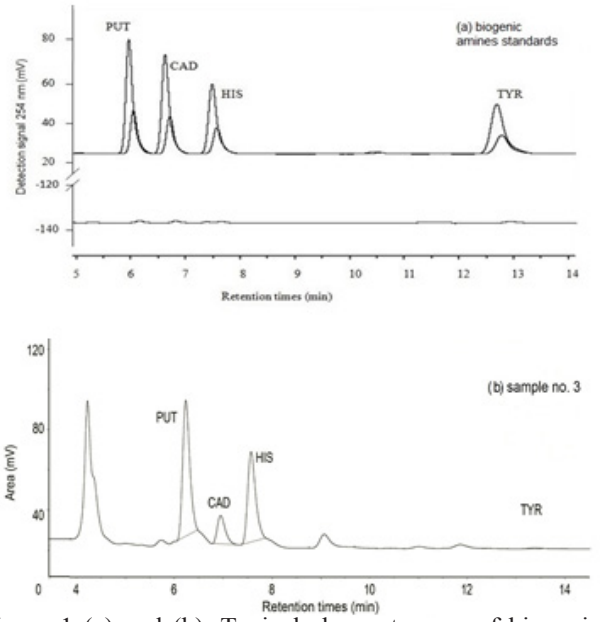

Figure 1 (a) and (b). Typical chromatogram of biogenic amines standards (0.1, 10, 50 mg L^{-1}) and belacan (sample no. 3).

below 50 mg kg^{-1}, the safe level in seafood products set by FDA USA (2001). The 50 mg kg^{-1} safe level is known not to produce any adverse effect to normal healthy individual as reported by FAO/WHO (2012). The highest histamine level in belacan product (sample no. 14) was found at 143 mg kg^{-1} with mean of 57.6 mg kg^{-1} (n=15). Histamine below 200 mg kg^{-1} is considered as acceptable quality for fermented products (EU, 2005). As for shrimp paste or fermented fish sauce, since it is consumed in small quantities (a few grams per serving), the recent Codex decisions (CODEX STAN 302 - 2011; Standard for Fish Sauce) have established that up to 400 mg kg^{-1} histamine in such products is safe for consumption.

Putrescine was moderately high with value ranging from 54-314 mg kg^{-1} (mean, 137 mg kg^{-1}) whereas the highest cadaverine found in belacan products was 134 mg kg^{-1} (mean, 50.1 mg kg^{-1}) and

Table 3. Percent recovery, intra-day repeatability (day 1) and inter-day repeatability assay (day 2, day 3 and day 6) measured as %RSD of spiked belacan sample with biogenic amines standards (1.0, 50, 100 mg L^{-1}).

Biogenic amines	Spiked concentration (mg L^{-1})	Recovery percentage (Day 1)	Intra-day Repeatability Day 1 (% RSD)	Inter-day repeatability (%RSD) Day 2	Day 3	Day 6
Putrescine	1	89.4	12.4	2.55	5.48	6.88
	50	114	2.09	3.91	3.39	8.25
	100	84.9	13.5	3.57	4.07	9.22
Cadaverine	1	73.1	4.11	4.61	4.87	6.13
	50	113	2.95	3.79	3.69	9.43
	100	89.2	17.4	3.68	4.37	8.82
Histamine	1	93.1	18.8	3.89	14.5	4.22
	50	103	3.65	3.87	3.83	7.89
	100	115	16.8	3.35	3.71	8.94
Tyramine	1	70.4	8.48	1.34	12.8	4.79
	50	97.4	5.74	4.12	5.56	13.9
	100	73.5	5.74	1.71	3.55	4.51

Table 4. Biogenic amines in belacan samples (Mean ± SD)

Sample Number	Concentration, mg kg^{-1} Putrescine	Cadaverine	Histamine	Tyramine	Total Biogenic Amines
Belacan 1	199 ± 3.20^{ef}	33.1 ± 1.31^{cd}	5.77 ± 0.93^{a}	13.3 ± 0.29^{f}	251 ± 3.2^{de}
Belacan 2	152 ± 0.14^{g}	53.5 ± 2.43^{f}	20.0 ± 0.35^{a}	20.0 ± 2.58^{a}	234 ± 7.10^{cde}
Belacan 3	83.4 ± 0.06^{bc}	24.8 ± 1.45^{bc}	50.1 ± 6.15^{b}	5.31 ± 0.12^{abc}	164 ± 7.69^{b}
Belacan 4	181 ± 6.36^{e}	47.1 ± 5.28^{ef}	22.9 ± 3.95^{a}	7.88 ± 0.02^{a}	259 ± 11.5^{e}
Belacan 5	87.2 ± 2.31^{c}	27.7 ± 1.48^{bc}	29.6 ± 5.45^{a}	5.82 ± 0.37^{bc}	150 ± 13.5^{b}
Belacan 6	96.6 ± 4.52^{c}	25.5 ± 6.58^{cd}	105 ± 8.13^{c}	4.74 ± 0.62^{ab}	232 ± 14.9^{cde}
Belacan 7	86.9 ± 6.51^{c}	30.3 ± 5.46^{bcd}	93.7 ± 4.38^{cd}	5.40 ± 0.08^{abc}	216 ± 16.9^{cd}
Belacan 8	217 ± 12.0^{f}	73.1 ± 6.88^{g}	3.86 ± 0.51^{a}	7.45 ± 0.90^{de}	301 ± 17.0^{f}
Belacan 9	216 ± 9.19^{f}	130 ± 7.0^{i}	112 ± 5.51^{e}	19.1 ± 1.15^{f}	471 ± 16.9^{g}
Belacan 10	54.0 ± 1.84^{a}	9.64 ± 1.06^{ad}	101 ± 12.8^{cd}	4.16 ± 0.17^{a}	169 ± 14.9^{b}
Belacan 11	63.9 ± 0.78^{ab}	9.56 ± 2.52^{a}	16.5 ± 3.65^{a}	5.99 ± 1.88^{bcd}	95.9 ± 3.26^{a}
Belacan 12	314 ± 11.5^{g}	134 ± 6.35^{i}	89.5 ± 8.06^{c}	12.1 ± 0.35^{f}	550 ± 14.4^{g}
Belacan 13	140 ± 7.07^{d}	40.9 ± 3.15^{de}	52.9 ± 6.05^{b}	6.69 ± 0.05^{cde}	241 ± 6.05^{de}
Belacan 14	61.8 ± 3.39^{a}	21.1 ± 5.58^{b}	143 ± 8.48^{e}	5.65 ± 1.72^{abc}	231 ± 11.9^{cde}
Belacan 15	92.4 ± 3.52^{c}	90.9 ± 5.42^{h}	15.5 ± 1.27^{a}	6.07 ± 0.60^{bcd}	205 ± 4.15^{cd}
Mean	137	50.1	57.6	8.64	243

Values are means ± S.D., values with different lowercase letters within the same column are significantly different ($p<0.05$) using one-way ANOVA.

tyramine 20.0 mg kg^{-1} (mean, 8.64 mg kg^{-1}). The tyramine level was unexpectedly low in all samples. Total biogenic amines are ranging from 95.9-550 mg kg^{-1} (mean, 243 mg kg^{-1}) with 20% of samples contain the total biogenic amines higher than the recommended level of 300 mg kg^{-1} (FDA USA, 2015). Results are shown in Table 4. The chromatogram of biogenic amines separation in belacan (samples no. 3) is shown in Figure 1(b).

Analysis using one-way ANOVA showed that biogenic amines are significantly different ($p<0.05$) between products. Despite high salt content (25-39.7% NaCl) used in many products (belacan 5, 7, 8, 12 and 15), high biogenic amines level (histamine, putrescine or cadaverine) were recorded in several products (belacan 7, 8 dan 12). Thus, biogenic amines formation in highly salted products were likely to be associated with the growth of halophilic and halotolerant bacteria. The accumulation of biogenic amines was believed to have occurred during the early stage of fermentation when the salt was not properly mixed or when bacterial decarboxylase enzymes are still active and free amino acids are available.

In semi solid fermentation process of belacan, improper mixing will produce non-homogenized dough with differences in the salt concentrations at certain localized area during fermentation. The effects of salt concentrations on saeu-jeot (salted shrimp fermentation) with 20%, 24%, 28% and 32% salt concentrations were studied by Se *et al.* (2014). The bacterial communities were monitored during the fermentation period. During the early fermentation period, *Vibrio, Photobacterium, Psychrobacter, Pseudo alteromonas* and *Enterovibrio* were identified as the dominant genera and the bacterial successions after 30-80 days fermentations were significantly different depending on the salt concentrations. *Salinivibrio* were predominant in the 20% and 24% salted samples, whereas *Staphylococus, Halomonas* and *Salimicrobium* were predominant in the 28% salted samples. This study suggests that 24-28% salt in saeu-jeot fermentation is appropriate for the production of safe and tasty saeu-jeot. Certain *Staphylococcus* sp. such as *S. epidermidis* and *S. capitis* are known as

prolific histamine forming bacteria producing 1000 and 400 μg mL^{-1} respectively (Hernåndez-Herrero *et al*. 1999) even in higher salt concentration. However, many factors are known to influence the growth of biogenic amines producing bacteria, in particular temperatures, pH, water activity, oxygen availability and salt concentrations (FAO/WHO 2012).

The effect of NaCl%, pH and moisture% on biogenic amines formation (histamine, cadaverine, putrescine and tyramine) in belacan products were tested using Pearson's correlation analysis. A significant correlation ($p< 0.05$) was found with NaCl% and cadaverine, pH with histamine, tyramine and putrescine levels ($p<0.05$). A statistical analysis also showed significant correlation ($p \leq 0.05$) between moisture% and cadaverine content in belacan products. Low moisture content (38.6%) in belacan sample number 11 may have resulted in low biogenic amines (histamine, cadaverine, putrescine and tyramine) in products despite low salt percentage (17.5%NaCl). The freshness of raw ingredient, proper temperature control and storage are among important factors that determine the microbial load responsible for biogenic amines accumulation in fermented products.

Conclusion

The levels of the important biogenic amines that are associated with food quality and safety assurance of local belacan products were determined. Local belacan products are still within the acceptable quality for safe consumption in fermented products (less than 200 mg kg^{-1}) based on the EU (2005) regulation guidelines for histamine. Histamine above 50 mg kg^{-1}, the safe limit with no observed side effect (FDA, 2001) was found in 53% of belacan products (means, 57.6 mg kg^{-1}). This finding suggests that the raw material is not fresh or deficiency in the cold storage whereas elevation in putrescine and cadaverine suggest decomposition process associated with microbial spoilage during processing or fermentation. There is a need to regulate and monitor the consistency in quality of local belacan. The simultaneous elevation of putrescine (mean, 137 mg kg^{-1}) and cadaverine (mean, 50.1 mg kg^{-1}) should raise a concern to consumer on food safety over the synergistic effect of histamine toxicity in the presence of both amines. Significant variations between products suggest lack of systematic approach in manufacturing procedures or hygienic practice in belacan production. Thus, regular monitoring on histamine and other biogenic amines content in food are beneficial as quality marker that could be valuable to consumers.

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