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Assessment of biogenic amine level from Cambodia fermented fish products

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Abstract

Food safety status has now been seen as a global concern as well as in Cambodia. Food allergies are one of the major problems, especially those caused by histamine. It is an important biogenic amine that can be found in fermented foods. This research study aimed to determine the biogenic amine levels and to isolate the histamine-producing bacteria in Cambodian fermented fish. A total of 20 samples of fermented fish products were randomly collected from four provinces in Cambodia, consisting of 11 samples of *prahok* (fish paste) and 9 samples of *toeuk-trey* (fish sauce). The histamine levels of seventeen samples and nine samples were lower than the Cambodian standard of 200 ppm and the standard FDA guideline of 50 ppm, respectively. The histamine-producing bacteria in *prahok* and *toeuk-trey* products were isolated and identified as *Staphylococcus piscifermentans* (five isolates), *Kocuria krisinae* (one isolate), *Staphylococcus pasteuri* (one isolate) and *Staphylococcus warneri* (one isolate). They were capable of producing 34.51 – 70.72 ppm of histamine contents in the culture broth. Therefore, the contamination of these histamine-producing bacteria could be related to the level of histamine. Thus, improper handling, processing and storage can be contributed to the cause of biogenic amines that could be hazardous in causing histamine poisoning.

1. Introduction

Fisheries products make a significant contribution to food security in Cambodia. In the daily diet of Cambodia, it comprises nearly 80% of the protein source. These products account for 8-12% of the country's GDP and can also be a key driver of poverty reduction. Of the up to 6 million people whose livelihoods depend on the fisheries sector, 87% are in small scale fishing, 9% in medium-scale fish, and 4% in large scale fishing (Baran, 2005; Teh *et al.,* 2019). The major products from fisheries are dried, smoke, sauces, and paste. Fermented fish products are very popular in Cambodia and are widely used as a food ingredient in Southeast Asia (Thaitawat *et al.*, 2000). In Cambodia, fish paste is called *prahok* and fish sauce is called *toeuk-trey.* These products are traditionally made by mixing fish with an amount of salt and fermented for six to twelve months or longer. Fermentation is an efficient way to preserve fish and improve flavour and nutrition by increasing the contents of amino acids, protein, unsaturated fats, and vitamins. In addition, fermentation also increases digestibility by separating proteins in fish and lowering the production of organic acids; and decreasing endogenous toxins (Sathe and Mandal, 2016).

Trey raw (*Channa marulius*), Trey kros (*Chitala chitala*), and Trey riel (*Cirrhinus caudimaculatus*) are common fish raw materials used in processing *prahok* (Norng *et al.*, 2011). Typically, this product is grey or brown in colour. Two common forms of *prahok*, bony *prahok* and boneless *prohok* are popular in Cambodia (Peng *et al.*, 2017). Today, most processors made *toeuk-trey* uses sea fish, but the homemade *toeuk-trey* in Cambodia uses fish caught in Tonle Sap Lake. The fermentation of these products includes microorganisms' digestion and self-digestion of fish materials. Fermented fish products are usually made by fishermen or smallholders in Cambodia. Farmers live in rural areas without electricity and clean water (Grace, 2015). Their production process is based on traditional methods, where there is a need to improve production hygiene standards. Fermented fish products are sensitive to contamination by microbial. These products are usually

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without labelled shelf-life and are stored at room temperature (Ly *et al.*, 2018). The quality of these products is impacted by handling, processing and storing. The pathogenic bacteria such as *Listeria* spp., *Salmonella* spp., *Escherichia coli*, *Bacillus*, *Enterobacter* spp., *Staphylococcus* spp., and *Clostridium* were found in fermented foods in Cambodia (Chuon *et al.*, 2014; Soeung *et al.*, 2015). Furthermore, there is no quality control system or surveillance program regarding microbiological and chemical contamination of fermented fish products in Cambodia. On the other hand, food safety is one of the national action plans of the Cambodian government (Mengchou and Spengler, 2016). Besides pathogenic bacteria concerns, biogenic amines could cause acute poisoning for consumers as well (Ruiz-Capillas and Herrero, 2019). In general, the content of biogenic amines has been found in fermented products, to be a significantly higher amount than in non-fermented food (Fardiaz and Markakis, 1979; Fukui *et al.*, 2012).

Histamine is one of the biogenic amines and a chemical hazard that causes scombroid poisoning. Scombroid poisoning has various symptoms such as nausea, urticarial, rashes, diarrhoea, itching of the skin, vomiting, tingling, and flushing (Lin *et al.*, 2014). In microbiological deterioration, histidine may produce a high level of histamine via decarboxylation (Shalaby, 1996; Kim *et al.*, 1999). Aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, 2 -phenylethylamine), and heterocyclic (histamine, tryptamine) are molecular biogenic amines. Some species of bacteria have histidine decarboxylase and are capable of producing histamines such as strains of *Raoultella planticola, Morganella morganii, Raoultella ornithinolytica,* and *Enterobacter aerogenes*; and strains of *Hafnia alvei, Vibrio alginolyticus, Citrobacter freundi,* and *Escherichia coli* (Takahashi *et al.*, 2003; Björnsdóttir-Butler *et al.*, 2010).

Prahok and *toeuk-trey* products are readily found in Cambodian markets. These kinds of products are considered to consume directly after the fermentation period without further cooking. Nowadays consumer demands are increasing for food safety and no chemical additives. The main purpose of this study was to determine the biogenic amines level and to isolate the histamine-producing bacteria in *prahok* and *toeuk-trey* products of Cambodia. The understanding of the safety quality of *prahok* and *toeuk-trey* products will be accomplished and protected the health of consumers.

2. Materials and methods

2.1 Samples

The sample of fermented freshwater fish products was collected from different processing places. Eleven *prahok* samples and nine *toeuk-trey* samples were collected from four provinces in Cambodia, namely Phnom Penh city (3 *prahok* and 2 *toeuk-trey*), Kandal province (3 *prahok* and 2 *toeuk-trey*), Kompong Chnang province (3 *prahok* and 2 *toeuk-trey*), and Kompot province (2 *prahok* and 3 *toeuk-trey*). These four provinces are potentially produced fermented fish products. *Prahok* and *toeuk-trey* samples were packaged in sterile plastic bags and put into sterile plastic bottles, then transported to the laboratory at Kasetsart University, Chalermphrakiat Sakonnakhon Province Campus, Sakonnakhon, Thailand. Samples were kept in a refrigerator at 4ºC.

2.2 pH, total acidity, and salt content determination

The method of the Association of Official Analytical Chemists (AOAC, 1995) was carried out to measure the value of pH, total acidity (TA), and salt content of each product. Ten grams of *prahok* sample were homogenized with 10 mL of distilled water and measured the pH of the sample using a pH meter. The pH of the *toeuk-trey* was measured directly on the sample by using the pH meter. The determination of total acidity (TA), *prahok* or *toeuk-trey* products (5 g or 5 mL) were homogenized with 50 mL of distilled water. The TA value was expressed as a percentage of lactic acid. For the salt content, *prahok* or *toeuk-trey* products (2 g or 2 mL) were homogenized in 18 mL of distilled water and titrated with 0.1 M $AgNO_3$ and 10% w/v K_2CrO_4 solution was used as the indicator.

2.3 Microbiological analysis

Prahok or *toeuk-trey* products were prepared with a ten-fold dilution with a diluent following the method of the Bacteriological Analytical Manual (FDA, 2001) (Table 1).

2.4 Biogenic amines analysis

The method of Riebroy *et al.* (2004) was carried out to determine histamine (Him), putrescine (Put), tryptamine (Trp), cadaverine (Cad), and tyramine (Tyr) from Sigma-Aldrich. The standards were separately dissolved in 0.1 M HCl (RCI Labscan, Thailand). To develop the standard curve, each standard stock solution was diluted to 1000 ppm, 800 ppm, 600 ppm, 400 ppm, 200 ppm, and 0 ppm.

Prahok or *toeuk-trey* products (10 g or 10 mL) were mixed with 50 mL of 10% trichloroacetic acid (TCA) solution by using a stomacher and then centrifuged at 4°

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Table 1. The culture media, incubation temperature and time for the different microorganisms.

Microorganisms	Media	Diluent/Enrichment Broth	Temperature (°C)	Time (hr)
Aerobic mesophilic bacteria	PCA agar	Peptone water	35	48
E. coli	EC broth	Butterfield's phosphate buffer (BPB)	44.5	24-48
Staphylococcus spp.	Baird-Parker agar	Butterfield's phosphate buffer (BPB)	35	24-48
Salmonella spp.	Bismuth Sulphite agar, Xylose Lysine Desoxycholate agar Hektoen agar	Tetrathonated broth	35	24
L. monocytogenes	Oxford agar	Listeria enrichment broth	35	48
Yeast and Mold	Dichloram Rose Bengal chloramphenicol (DRBC)	Peptone water	25	120

C for 20 mins at 8000×*g*. The supernatant (250 µL) was mixed with 75 µL of saturated sodium bicarbonate solution and 50 µL of 2 M NaOH solution. Next, added 500 µL of dansyl chloride solution (diluted in acetone, the concentration of 10 mg/mL) to the mixture, and incubated at 40°C for 45 mins. After that, added 25 µL of 25% ammonia to stop the reaction. The mixture was centrifuged at 4°C for 30 mins at 3000×*g*. Next, filtered the supernatant with a 0.45 µm nylon membrane filter, and injected 20 µL of the filtrate into the HPLC system (Agilent Technology, Germany). A mixture of 0.1% acetic acid in acetonitrile (B) and 0.1% acetic acid in water (A) was used as the mobile phase. The gradient program started with 50% B and 50% A (0 min) (flow rate of 1 mL/min); after that, solvent B was increased to 90% within 25 min (1 mL/min); then switched to the gradient system to 50% B and 50% A within 10 min (1 mL/min), and held for 5 mins. The wavelength of UV detection was set at 254 nm and the temperature of a C18 column (5 µm × 4.6 mm × 250 mm) was set at 40ºC.

The series dilution of each biogenic amine standard was analyzed along with test samples. The standard curves were created by the peak areas from the injection of biogenic amines standard solutions, and then the standard curve equations were used to determine biogenic amines concentration in *prahok* and *toeuk-trey* products.

2.5 Isolation of histamine-producing bacteria

Prahok or *toeuk-trey* products (10 g or 10 mL) were homogenized with 90 mL of 0.1% peptone water. Niven's agar, supplemented with L-histidine, was used to isolate histamine-producing bacteria by incubating for 4 days at 35°C. The colonies with blue or purple colour were selected and streaked on tryptic soy agar (TSA). Tryptic soy broth (TSBH), fortified by 1% L-histidine, was used to culture isolated bacteria by incubating for 24 hours at 35°C without shaking. Then, the culture broth (TSBH) (250 µL) was taken for the quantity of biogenic amines (Niven *et al.*, 1981). Gibthai Co., Ltd (Thailand) was carried out to identify the pure isolates species. Amplification of the 16S rDNA of histamine-producing bacteria was performed using sequencing primer 785F 5' (GGA TTA GAT ACC CTG GTA) 3', 907R 5' (CCG TCA ATT CMT TTR AGT TT) 3, and PCR primer 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3, 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'.

2.6 Statistical analysis

Data analysis of samples was carried out using a t-test and a completely randomized design (CRD). Pearson's correlation was carried out to determine relationships between pH, total acidity, salt content, APC, *Staphylococcus* spp., and histamine contents in the fermented fish products. A value of $P < 0.05$ was used to indicate significant deviation. All statistical analysis used the SPSS version 21.0 for windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Physicochemical characteristics in fermented fish products

Table 2 shows the values of pH, total acidity (TA), and salt content in *prahok* and *toeuk-trey* products ranging from 3.68 – 6.42, 0.16 – 2.68% and 14.70 – 36.77%, respectively. The average values of the pH, TA and salt content in *prahok* were 5.97, 1.69% and 25.03%, respectively. The average values of pH, TA, and salt content in *toeuk-trey* were 4.16, 0.25% and 21.34%, respectively. In *prahok* samples, the average values of pH, total acidity and salt content were slightly higher than *toeuk-trey* samples. These findings were consistent with the reports on fermented fish previously published in Thailand, China and Taiwan (Riebroy *et al.*, 2004; Tsai *et al.*, 2006; Zeng *et al.*, 2013; Rattanasena and Chaikham, 2018).

3.2 Microorganisms in fermented fish products

The values of APC, *Staphylococcus* spp., yeast and mould, *Salmonella* spp., *Listeria monocytogenes* and *E.*

Table 2. Values of pH, total acidity (TA), and salt content in *prahok* and *toeuk-trey* products.

Province	Sample code	Parameters		
		pH	TA (%)	Salt content (%)
Phnom Penh	PP-P-1	6.40±0.01[a]	2.68±0.12[a]	24.27±0.10[a]
	PP-P-2	5.66±0.00[c]	1.28±0.13[b]	22.47±0.20[b]
	PP-P-3	5.81±0.00[b]	1.52±0.01[b]	20.15±0.74[c]
	PP-S-1	4.65±0.00[a]	0.33±0.01[a]	28.10±0.70[a]
	PP-S-2	3.78±0.00[b]	0.17±0.01[b]	14.81±0.15[b]
Kandal	KDL-P-1	6.09±0.01[a]	0.75±0.03[b]	31.45±0.54[a]
	KDL-P-2	6.02±0.01[b]	1.53±0.16[a]	19.77±0.59[c]
	KDL-P-3	5.84±0.00[c]	1.67±0.07[a]	22.30±0.55[b]
	KDL-S-1	4.15±0.01[b]	0.29±0.01[ns]	18.69±0.34[b]
	KDL-S-2	4.69±0.00[a]	0.31±0.01[ns]	28.47±0.34[a]
Kompong Chnang	KPC-P-1	5.78±0.01[c]	0.87±0.07[b]	28.96±0.24[a]
	KPC-P-2	6.02±0.00[a]	2.70±0.01[a]	28.31±0.81[a]
	KPC-P-3	5.98±0.00[b]	0.93±0.02[b]	23.76±0.35[b]
	KPC-S-1	3.88±0.00[b]	0.16±0.02[ns]	14.77±0.19[b]
	KPC-S-2	4.66±0.00[a]	0.30±0.01[ns]	28.35±0.21[a]
Kompot	KP-P-1	5.88±0.01[b]	2.76±0.17[ns]	36.77±0.61[a]
	KP-P-2	6.16±0.00[a]	1.92±0.08[ns]	17.18±0.23[b]
	KP-S-1	4.10±0.00[a]	0.34±0.01[a]	18.65±0.28[b]
	KP-S-2	3.68±0.01[c]	0.17±0.00[b]	25.62±0.16[a]
	KP-S-3	3.85±0.01[b]	0.19±0.01[b]	14.71±0.40[c]

Values were presented as mean±SD. Values with different superscript within the same row of each province are significantly different at $P < 0.05$. P = fish paste, S = fish sauce, [ns]No significant difference.

coli in *prahok* and *toeuk-trey* samples are presented in Table 3. The values of APC and *Staphylococcus* spp. ranged from <1 – 7.20 log CFU/g and <1 – 4.41 log CFU/g, respectively, in all samples. For *prahok* samples, the average values of APC (5.50 log CFU/g) and *Staphylococcus* spp. (3.99 log CFU/g) were significantly ($P < 0.05$) larger than the *toeuk-trey* samples. The pathogenic bacteria, including *Salmonella* spp., *E. coli* and yeast and mould, were not found in any sample; while *L. monocytogenes* were found in one sample of *prahok* samples. No significant differences ($P > 0.05$) was found between *Salmonella* spp., *E. coli,* yeast and mould and *L. monocytogenes.* The Cambodian Standards guidelines CS (2015) suggested that there should be no *L. monocytogenes* per 25 g of sample, no *Salmonella* spp. per 25 g of sample, less than 3 MPN of *E. coli* per 1 g sample and yeast and mould less than 50 CFU/g. Therefore, the detected rate of unacceptable fermented fish products was 5% (1/20) for *L. monocytogenes*, based on the Cambodian regulatory standards. Achinewhu and Oboh (2002), and Kose and Hall (2011) reported that salt concentration could be a significant impact on the growth and fermentation period of microorganisms. *Prahok* and *toeuk-trey* products were made by removing the head and scales, soaking them in the water and then storing them at room temperature, so that microorganisms can contaminate the fish and easily multiply. Thus, unsanitary handling, processing, and storage of fermented fish products resulted in poor microbiological quality in these products.

3.3 Biogenic amines level in fermented fish products

Biogenic amines contents of *prahok* and *toeuk-trey* products are listed in Table 4. The values of Try, Put, Cad, His and Tyr in samples ranged from 17.94 – 139.65 ppm, 9.94 – 558 ppm, 9.22 – 540 ppm, 5.40 – 368 ppm and 0.86 – 124 ppm, respectively. The average values of Try, Put, Cad, His and Tyr in *prahok* samples were 100.70 ppm, 253.31 ppm, 293.31 ppm, 148.21 ppm and 40.78 ppm, respectively. The average values of Try, Put, Cad, His and Tyr in *toeuk-trey* samples were 36.63 ppm, 54.14 ppm, 56.01 ppm, 31.97 ppm and 15.06 ppm, respectively. The average values of Try, Put, Cad and His in *prahok* were significantly ($P < 0.05$) greater than for *toeuk-trey*, because *toeuk-trey* products were boiled during processing that killed biogenic amines-producing bacteria; while non-significantly different ($P > 0.05$) was found for Tyr. Fardiaz and Markakis (1979) indicated that biogenic amines contents were usually detected in high concentrations in fermented fish products. For example, histamine levels in fish paste, fish sauce and shrimp paste ranged from 101 to 760 ppm, 45 to 1220 ppm and 20 to 760 ppm, respectively (Tsai *et al.*, 2006). Rattanasena and Chaikham (2018) also reported histamine at 118.42 to 163.92 mg/kg in *Plaa-Som* product. On the other hand, Try, Put, Cad and Tyr were

Table 3. Values of APC, *Staphylococcus* spp., yeast and mold, *Salmonella* spp., *Listeria monocytogenes* and *E. coli* in *prahok* and *toeuk-trey* products.

Province	Products	Sample code	Parameters					
			APC (log CFU/g)	*Staphylococcus* spp.(log CFU/g)	Yeast and mold^{ns} (log CFU/g)	*Salmonella* spp.	*L. monocytogenes*	*E.coli*^{ns} (MPN/g)
Phnom Penh	Fish paste	PP-P-1	6.44±0.03^{b}	4.19±0.08^{a}	<1	–	–	<1
		PP-P-2	5.04±0.06^{c}	4.35±0.10^{a}	<1	–	–	<1
		PP-P-3	7.02±0.04^{a}	3.93±0.07^{b}	<1	–	–	<1
	Fish sauce	PP-S-1	4.16±0.06^{ns}	<1	<1	–	–	<1
		PP-S-2	4.13±0.04^{ns}	<1	<1	–	–	<1
Kondal	Fish paste	KDL-P-1	4.45±0.02^{b}	3.97±0.08^{b}	<1	–	–	<1
		KDL-P-2	4.08±0.01^{c}	4.41±0.06^{a}	<1	–	–	<1
		KDL-P-3	6.36±0.12^{a}	3.22±0.18^{c}	<1	–	–	<1
	Fish sauce	KDL-S-1	4.37±0.09^{ns}	3.85±0.10	<1	–	–	<1
		KDL-S-2	3.52±0.07^{ns}	<1	<1	–	–	<1
Kompong Chnang	Fish paste	KPC-P-1	5.24±0.02^{c}	3.93±0.10^{b}	<1	–	–	<1
		KPC-P-2	5.45±0.08^{b}	3.83±0.09^{b}	<1	–	–	<1
		KPC-P-3	5.69±0.05^{a}	4.18±0.07^{a}	<1	–	+	<1
	Fish sauce	KPC-S-1	<1	<1	<1	–	–	<1
		KPC-S-2	<1	<1	<1	–	–	<1
Kompot	Fish paste	KP-P-1	5.24±0.41^{ns}	4.40±0.09^{a}	<1	–	–	<1
		KP-P-2	5.27±0.13^{ns}	3.45±0.22^{b}	<1	–	–	<1
	Fish sauce	KP-S-1	<1	<1	<1	–	–	<1
		KP-S-2	3.85±0.07	<1	<1	–	–	<1
		KP-S-3	<1	<1	<1	–	–	<1

Values were presented as mean±SD. Values with different superscript within the same row of each province are significantly different at $P < 0.05$. ^{ns}No significant difference.

Table 4. Values of biogenic amines in *prahok* and *toeuk-trey* products.

Province	Products	Sample code	Biogenic amines (ppm)				
			Tryptamine	Putrescine	Cadaverine	Histamine	Tyramine
Phnom Penh	Fish paste	PP-P-1	139.65±5.37^{a}	370.59±1.49^{a}	502.52±11.40^{a}	208.85±0.06^{a}	23.81±2.19^{b}
		PP-P-2	102.17±1.87^{b}	106.36±6.20^{c}	90.83±6.28^{c}	26.12±1.96^{c}	33.86±0.05^{a}
		PP-P-3	95.35±0.19^{b}	123.30±0.05^{b}	134.45±1.40^{b}	42.41±0.04^{b}	20.90±0.34^{b}
	Fish sauce	PP-S-1	18.90±2.65^{b}	49.05±2.75^{b}	20.45±4.47^{b}	16.71±0.07^{b}	0.86±0.36^{b}
		PP-S-2	54.30±2.99^{a}	137.68±2.71^{a}	137.92±2.98^{a}	75.93±0.23^{a}	48.15±0.34^{a}
Kondal	Fish paste	KDL-P-1	83.42±2.03^{b}	203.26±4.05^{a}	221.77±6.29^{a}	120.54±11.16^{a}	43.27±0.79^{a}
		KDL-P-2	80.67±3.80^{b}	111.36±1.27^{c}	112.66±9.89^{b}	27.69±1.96^{c}	19.60±3.72^{b}
		KDL-P-3	100.97±1.49^{a}	122.57±0.94^{b}	131.71±1.28^{b}	97.58±0.60^{b}	13.11±0.60^{c}
	Fish sauce	KDL-S-1	30.89±3.91^{b}	27.88±1.34^{b}	17.56±2.54^{b}	10.33±0.99^{b}	6.82±0.60^{b}
		KDL-S-2	57.92±0.33^{a}	132.63±2.64^{a}	132.18±3.67^{a}	73.74±0.01^{a}	34.83±4.88^{a}
Kompong Chnang	Fish paste	KPC-P-1	71.86±5.56^{b}	173.85±3.37^{c}	208.38±2.19^{c}	166.93±3.11^{b}	87.18±0.16^{a}
		KPC-P-2	112.03±0.74^{a}	330.61±1.38^{b}	468.11±2.09^{a}	203.61±0.32^{a}	20.05±0.40^{c}
		KPC-P-3	80.48±0.85^{b}	376.54±2.57^{a}	351.56±3.33^{b}	171.52±0.02^{b}	38.56±0.12^{b}
	Fish sauce	KPC-S-1	17.94±0.40^{b}	39.66±3.64^{ns}	19.46±0.17^{b}	10.90±0.35^{b}	1.09±0.08^{b}
		KPC-S-2	58.46±2.07^{a}	37.21±0.64^{ns}	131.58±0.73^{a}	73.93±0.31^{a}	34.07±0.60^{a}
Kompot	Fish paste	KP-P-1	119.53±1.49^{ns}	309.61±2.39^{b}	434.62±0.28^{b}	196.46±5.85^{b}	23.98±3.25^{b}
		KP-P-2	114.62±1.23^{ns}	558.38±1.73^{a}	540.82±2.31^{a}	368.58±3.65^{a}	124.98±5.50^{a}
	Fish sauce	KP-S-1	29.48±4.30^{b}	38.12±0.40^{a}	17.00±0.04^{a}	9.76±0.06^{b}	6.00±0.01^{a}
		KP-S-2	43.71±2.52^{a}	15.03±0.46^{b}	9.22±2.87^{b}	5.04±0.05^{c}	2.80±0.21^{b}
		KP-S-3	18.08±0.20^{c}	9.94±0.76^{c}	18.75±0.26^{a}	11.00±0.10^{a}	0.96±0.03^{c}

Values were presented as mean±SD. Values with different superscript within the same row of each province are significantly different at $P < 0.05$. ^{ns}No significant difference.

Table 5. Histamine distribution in 11 *prahok* products and 9 *toeuk-trey* products.

Histamine level (ppm)	*Prahok* (%)	*Toeuk-trey* (%)	*Prahok* and *toeuk-trey* (%)
<49	3 (27.27)	6 (66.67)	9 (45.00)
50-99	1 (9.10)	3 (33.33)	4 (20.00)
100-199	4 (36.36)	0 (0.00)	4 (20.00)
>200	3 (27.27)	0 (0.00)	3 (15.00)
Total	11	9	20

also detected in *prahok* and *toeuk-trey* products. Try, Put, Cad and Tyr could also cause health hazards with their high concentrations. Try, Put, Cad, and Tyr have been indicted as spoilage of the products and increased their concentrations with longer storage time (Galgano *et al.*, 2009; Gardini *et al.*, 2016). The research study of Biji *et al.* (2016) reported that Try, Put, Cad and Tyr can cause acute toxicity with a concentration greater than 2000 ppm. Nevertheless, Try, Cad and Put had pharmacological activities less effect than His and Tyr. The research of Tsai *et al.* (2006) found that the levels of Put, Cad, and Tyr in fish sauce ranged from 2.0 to 243 ppm, ND to 243 ppm and ND to 42 ppm, respectively; and the levels of Put, Cad and Tyr in fish paste ranged from 5.0 to 17 ppm, 22 to 107 ppm and ND to 32 ppm, respectively. Riebroy *et al.* (2004) stated that biogenic amines detected in samples could be used as important indicators of bacterial contamination. Good hygienic practice and Good manufacturing practice could remove the presence of bacteria and minimize the aggregation of biogenic amines in *prahok* and *toeuk-trey* products.

The histamine levels distribution in *prahok* and *toeuk -trey* products are shown in Table 5. Approximately 55% (11/20) of *prahok* and *toeuk-trey* products contained histamine content exceeding 50 ppm, which is the limit allowed by USFDA (2001) and 85% (17/20) samples had histamine levels less than Cambodian regulation standards of 200 ppm (CS, 2015). The Centers for Disease Control and Prevention CDC (2000) confirmed that scombroid poisoning occurred when the histamine level was 200 ppm. However, histamine contents greater than 100 ppm could be toxic and insecure for consumers (Bartholomew *et al.*, 1987). Among the 20 samples of this study, seven samples (35%) had histamine contents greater than 100 ppm (Table 5). Referring to the toxicological histamine contents information in fermented fish products that can be a health hazard, consumption of these seven samples may cause disease symptoms, due to their excessive histamine contents. The high level of histamine was found in *prahok* and *toeuk-trey* products, however, only a few reports of foodborne histamine poisoning have been reported because of the consumption of fermented fish products, and in Cambodia, there have been no reports about foodborne histamine intoxication. Furthermore, Taylor and Eitenmiller (1986) revealed that foodborne histamine intoxication was not reported, although in the countries that have a tracking records system. Hence, it is very important for consumers to realize that *prahok* and *toeuk -trey* products can be harmful and caused histamine poisoning.

The positive correlation parameters which statically significant correlation were existed between pH and TA (r = 0.81, P < 0.05), pH and salt content (r = 0.42, P < 0.05), pH and APC (r = 0.73, P < 0.05), pH and *Staphylococcus* spp. (r = 0.91, P < 0.05), pH and histamine (r = 0.67, P < 0.05), TA and salt content (r = 0.34, P < 0.05), TA and APC (r = 0.64, P < 0.05), TA and *Staphylococcus* spp. (r = 0.74, P < 0.05), TA and histamine (r = 0.67, P < 0.05), APC and *Staphylococcus* spp. (r = 0.74, P < 0.05), APC and histamine (r = 0.48, P < 0.05), and *Staphylococcus* spp. and histamine (r = 0.50, P < 0.05). The positive relationship parameters which significantly different were existed between salt content and APC (r = 0.14, P > 0.05) salt content and *Staphylococcus* spp. (r = 0.42, P > 0.05) and salt content and histamine (r = 0.27, P > 0.05). According to the study of Gardini *et al.* (2016) reported that pH, salt content, microbiological and biogenic amines had strongly positive correlation. Increasing salt concentrations in fermented fish products reduced bacteria growth and biogenic amines accumulation. In contrast, Tsai *et al.* (2006) reported that no significant correlation existed between pH, salt content, APC and histamine.

3.4 Histamine-producing bacteria in fermented fish products

Histamine-producing bacteria identification from *prahok* and *toeuk-trey* products in Cambodia and biogenic amines production in the culture broth are presented in Table 6. On HBI agar plates, the tested samples produced 29 purple and blue colonies. The isolates colonies were steaked on trypticase soy agar (TSA) following inoculation in TSB fortified with 1.0% L-histidine, with 8 out of 29 isolates showing the capacity to produce histamine in the culture broth. Eight isolates of bacteria were analyzed for their 16S rDNA sequences. Eight isolate bacteria were identified as *Staphylococcus piscifermentans* (five strains), *Kocuria krisinae* (one strain), *Staphylococcus pasteuri* (one strain), and *Staphylococcus warneri* (one strain) with the sequences confirmed from 99 to 100% homology, and

Table 6. Histamine-producing bacteria identification from *prahok* and *toeuk-trey* products in Cambodia and biogenic amines production in the culture broth.

Strain ID	Strain species	% Identity	Biogenic amines (ppm)				
			Tryptamine	Putrescine	Cadaverine	Histamine	Tyramine
H-PP-P-1-1	*Staphylococcus piscifermentans*	99	ND*	ND	60.56±7.77	40.41±3.27	24.28±0.96
H-KDL-P-1-1	*Staphylococcus piscifermentans*	100	ND	ND	58.98±7.75	46.30±11.49	26.42±1.44
H-KDL-S-2-1	*Kocuria krisinae*	99	ND	ND	50.80±1.07	43.27±0.13	27.05±4.36
H-KPC-P-1-1	*Staphylococcus warneri*	99	ND	ND	60.43±2.40	70.72±18.65	25.25±3.20
H-KPC-P-2-3	*Staphylococcus piscifermentans*	99	ND	ND	49.29±0.71	43.86±10.32	32.53±3.33
H-KP-P-1-2	*Staphylococcus piscifermentans*	99	ND	ND	53.11±2.88	34.51±0.81	34.00±4.48
H-KP-P-2-1	*Staphylococcus piscifermentans*	100	ND	ND	62.55±17.85	37.07±4.74	25.16±5.79
H-KP-P-2-2	*Staphylococcus pasteuri*	99	ND	ND	59.11±1.61	42.68±9.62	28.81±6.65

ND, not detected (under the detection limit).

were capable of producing 34.51 to 46.30 ppm, 43.27 ppm, 42.68 ppm, and 70.72 ppm of histamine contents in the culture broth, respectively. These isolate strains also produced Cad and Tyr. The *S. piscifermentans* strains produced Cad and Tyr in TSBH ranged from 49.29 to 62.55 ppm and 24.28 to 34.00 ppm, respectively. In TSBH, *K. krisinae* strain produced 50.80 ppm of Cad and 27.05 of Tyr; *S. warneri* strain produced 60.43 ppm of Cad and 25.25 ppm of Tyr; and *S. pasteuri* strain produced 59.11 ppm of Cad and 28.81 ppm of Tyr in the culture broth.

In this research, most of the isolates bacteria that produce histamine belonged to *Staphylococcus* spp. such as *S. piscifermentans*, *S. warneri,* and *S. pasteuri*. Yatsunami and Echigo (1993) mentioned that *Staphylococcus* spp., *Pseudomonas*, and *Vibrio* spp. were the most recorded bacteria that produced histamine, contributing almost 50% of histamine-producing microorganism and having to have strong activities in producing histamine in fermented salted fish. However, the currently isolated *S. xylosus, S. sciuri* and *S. pasteuri* were recorded as weak histamine-producers (Kung *et al.*, 2007; Hsu *et al.*, 2009). Similarly, *S. piscifermentans*, *S. warneri* and *S. pasteuri*, which were also poor histamine producers, able to produce 34.51 to 46.30 ppm of histamine in the culture broth. The bacteria can be transmitted to fermented fish products through noticeable human interaction during handling, processing or storage. Although *K. krisinaes* has been rarely reported as a histamine-producer, it was a crucial strain that produced histamine in this research, accounting for 12.5% of the overall histamine-producing strains. *K. krisinaes* has been isolated from 'kodong sufu' and recorded as a pathogen causing human infection (Chen *et al.*, 2015; Lv *et al.*, 2020). Moreover, the main histamine -producing strains in fish species were not identified in this research; for example *M. morganii, E. aerogenes, , R. ornithinolytica, P. damselae, H. alvei, C. freundi, V. alginolyticus*, and *R. planticola* (Takahashi *et al.*, 2003; Björnsdóttir-Butler *et al.*, 2010).

4. Conclusion

In this research, the values of microorganisms and pathogenic bacteria in fermented fish products were safe for consumers; except one *prahok* sample presented *Listeria monocytogenes*. Three samples of fermented fish products had histamine levels that exceeded the Cambodian standard of 200 ppm, while 11 samples exceeded the FDA guideline of 50 ppm. There are very important for consumers to be aware of the hazards of causing histamine poisoning. Additionally, *S. piscifermentans, S. warneri, S. pasteuri* and *K. krisinae* were identified as histamine-producing bacteria in *prahok* and *toeuk-trey* products, indicating that unsanitary handling, processing and storage could lead to the accumulation of biogenic amines in the products. These findings indicate that the processors should be applied to Good Hygienic Practice (GHP), Good Manufacturing Practice (GMP), and HACCP in the future processing of fermented fish products to ensure their safety for human consumption.

Conflict of interest

The authors declare no conflict of interest.

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