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Occurrence of Histamine and Histamine-forming Bacteria in Philippine Traditional Dried-salted Fish Products

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Abstract

Twenty-one dried-salted fish products sold at major retail markets in the province of Iloilo, Philippines were purchased and tested to determine the occurrence of histamine and histamine-forming bacteria. The levels of pH, salt content, moisture content, water activity (Aw), total volatile basic nitrogen (TVBN), aerobic plate count (APC), *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* in all samples ranged from 6.36 to 6.71, 2.55 % to 15.84 %, 21.12 % to 49.99 %, 0.67 to 0.87, 19.25 to 69.05 mg.100 g^{-1}, 3.0 to 7.39 log CFU.g^{-1}, <3 to 28 MPN.g^{-1}, absent in 25 g and 3.58 to 6.89 log CFU.g^{-1}, respectively. Ten (47.6 %) dried fish samples had histamine levels greater than the United States Food and Drug Administration guideline of 5 mg.100 g^{-1} for scombroid fish and/or scombroid products, whereas seven (33.3 %) samples contained histamine levels greater than 20 mg.100 g^{-1} which is sufficient to cause the symptoms of scombroid poisoning according to the Centers for Disease Control and Prevention. Seven histamine-forming bacterial strains were isolated and identified biochemically and morphologically as belonging to genus *Vibrio*, *Salmonella*, *Staphylococcus*, *Bacillus* and *Pseudomonas*. The presence of these bacteria in dried fish is indicative of poor standards of process hygiene and sanitation as well as mishandling during storage.

Keywords: histamine, histamine-forming bacteria, dried-salted fish, scombroid poisoning

Introduction

Histamine or scombroid poisoning is a common cause of foodborne disease particularly in association with the ingestion of mishandled scombroid fish belonging to the families Scombridae (e.g. tuna and mackerel) and Scomberesocidae (e.g. saury) (Taylor 1986). These fish species usually contain high levels of free histidine in their muscle and are implicated scombroid poisoning.

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Although several species of non-scombroid fish such as mahi-mahi, bluefish, herring and sardines are also involved in incidents of scombroid poisoning as well as in dried and fermented fish products (Lehane and Olley 2000; Mah et al. 2002; Lin et al. 2012). A variety of symptoms including rash, urticaria, nausea, vomiting, diarrhea, flushing, and tingling and itching of the skin is often associated with histamine poisoning (Taylor 1986). Symptom severity is dependent on the amount of histamine ingested and the individual's sensitivity to histamine (Russell and Maretic 1986).

The decarboxylation of specific free amino acids by exogenous decarboxylases released by microbial species associated with seafood contributes to the formation of biogenic amines in many fish and fishery products. Various bacterial species of the Enterobacteriaceae family are known to contain histidine decarboxylase and have the ability to produce histamine (Huang et al. 2010). The species included *Morganella (Proteus) morganii*, *Klebsiella pneumoniae*, *Hafnia alvei*, *Proteus vulgaris*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Serratia fonticola*, *Serratia liquefaciens*, *Raoultella* (formerly *Klebsiella*) *planticola*, *R. ornithinolytica*, *Providencia stuartii* and *Citrobacter freundii* (Ababouch et al. 1991; López-Sabater et al. 1994; Kim et al. 2001, 2003; Tsai et al. 2006). Other bacterial species such as *Clostridium* spp., *Vibrio alginolyticus, Acinetobacter lowffi, Plesiomonas shigelloides, Pseudomonas putida, Pseudomonas fluorescens, Aeromonas* spp., and *Photobacterium* spp. have also been reported as histamine producers (Yatsunami and Echigo 1991; Okuzumi et al. 1994; Lopez-Sabater et al. 1996).

Salt-drying is a traditional method of fish preservation that involves several steps such as back-cutting, degutting, salting and sun-drying for several days. The final product results into a hard consistency with low water activity (Aw, 0.75) and high salt content (5–25 %) (Huang et al. 2010). But high levels of histamine have often been detected in processed fish products in Southeast Asian countries (Kanki et al. 2004; Tsai et al. 2006, 2007; Huang et al. 2010). The high level of histamine can be attributed to various factors contributing to histamine development along the supply chain, such as temperature abuse during handling of fresh fish, post processing contamination, improper packaging and storage in the retail outlets.

In the Philippines, dried-salted fish is one of the ethnic products and is widely consumed locally. In fact, Iloilo province is one of the leading producers of dried fish products in the country (BFAR 2013). But the possible incidents of histamine fish poisoning were normally unreported, or they may have occurred but gone unnoticed because symptoms closely resemble those of food allergies (Lin et al. 2012). The "itchy" or biting mouth sensation upon consumption of dried fish is an indication of histamine occurrence. High levels of histamine can be a food-borne chemical hazard and it is a public safety concern. There has been no report on the presence of biogenic amines, including histamine, histamine-forming bacteria, and related bacteria in traditional dried-salted fish products in the Philippines. Therefore, this study analysed 21 major dried-salted fish products sold in retail markets in Iloilo province to provide a safety quality assessment of these products.

Materials and Methods

Samples

Twenty one major dried-salted fish products were purchased from retail markets in Iloilo province, Philippines in August and September 2015. The top three fish species used in dried-salted fish production and mostly histamine-formers were chosen for analysis namely Indian mackerel *Rastrelliger kanagurta* (Cuvier 1816) (seven samples), sardines *Sardinella* spp. (seven samples) and anchovies *Stolephorus* spp. (seven samples).

Market samples of *Sardinella* spp. were a combination of *Sardinella longiceps* Valenciennes 1847, *Sardinella fimbriata* (Valenciennes 1847), and *Sardinella gibbosa* (Bleeker 1849), hence, designated in this study as *Sardinella* spp. While anchovies were a combination of *Stolephorus commersonnii* Lacepède 1803 and *Stolephorus indicus* (van Hasselt 1823), hence, designated in this study as *Stolephorus* spp. In general, the dried fish products were unpacked and maintained at ambient temperature in the retail stores prior to purchase. All samples were wrapped in aseptic polyethylene bags, placed in ice and immediately transported to the laboratory for use within 4 h.

Determination of pH value, salt content, moisture content, water activity and total volatile base nitrogen

Dried fish samples (5 g) were homogenised in a mortar and pestle with 50 mL triple distilled water for 1 min at ambient temperature and the pH values of the homogenates were measured using a calibrated digital pH meter (Orion™ Thermo Scientific, USA). Salt content was determined by homogenising 2 g of dried fish sample with 18 ml of distilled water (AOAC 2005). The homogenate was titrated with 0.1 M silver nitrate ($AgNO_3$, Sigma, USA) using 10 % w/v potassium chromate (K_2CrO_4, Sigma, USA) solution as an indicator. The moisture content was conducted with the standard gravimetric method by drying 1–3 g of a test sample at 102.0 °C ± 2.0 ℃ under atmospheric pressure for 2 h.

The consistency of mass was tested by additional drying steps of 1 h until the difference in mass did not exceed 0.5 mg (AOAC 2005). Water activity (Aw) was determined by an Aw meter (Novasina ms1-aw, Switzerland) at 27 °C. The total volatile base nitrogen (TVBN) content was measured by Conway's microdiffusion method (Cobb et al. 1973) in which TVBN extract of the fish sample was mixed in 6 % trichloroacetic acid (Sigma, USA), then absorbed by boric acid (Sigma, USA) and titrated with 0.02N hydrogen chloride (HCl, Sigma, USA). The TVBN content was expressed in mg.100 g^{-1} fish.

Microbiological analysis

Aerobic plate count (APC) was measured by homogenising a 30 g sample into a 270 mL sterile peptone broth.

Serial dilution was prepared by pipetting 1 mL of each decimal dilution sterile plates. Nutrient agar (Difco, USA) supplemented with 3 % sodium chloride (NaCl, Sigma, USA) was poured, allowed to set and incubated at 35–37 °C for 24–48 h. APC was expressed as $\log_{10}$ colony forming units (CFU) g^{-1}.

Most probable number (MPN) method was used in the analyses of total coliforms (TCs) and *Escherichia coli* in the dried fish samples (U.S. Food and Drug Administration 1998). Lauryl sulfate tryptose (LST) broth (Difco, USA) and brilliant green lactose bile (BGLB) (2 %) broth (Difco, USA) incubated at 35 °C for 48 h were used for presumptive and confirmation tests for TCs, respectively. *Escherichia coli* was determined using the LST broth and *E. coli* (EC) broth (Difco, USA) incubated at 35 °C and 44 °C, respectively. Positive gas production in EC broth were then confirmed by eosin methylene blue agar (EMBA) (Difco, USA) and by indole, methyl red, Voges-Proskauer and citrate (IMViC) tests. For *Salmonella typhi* determination, a 25 g sample was homogenised with 225 mL pre-enrichment broth (lactose broth) and incubated at 35 °C for 24 h. One mL of the pre-enrichment broth was transferred into tubes containing tetrathionate broth (TTB, Difco, USA) and incubated at 35 °C for 24 h.

Enriched cultures were streaked in xylose lysine deoxycholate (XLD) agar (Difco, USA) and incubated at 35 °C for 24 h. Typical *Salmonella* sp. colonies were screened through biochemical test on triple sugar iron (TSI, Difco, USA) and IMViC tests. While for *Staphylococcus aureus*, direct plate count method was used wherein a 25 g sample was homogenized in 225 mL of 0.1 % peptone with added 1 % NaCl. Serial dilution was prepared by equally distributing 1 mL sample suspension into 3 plates of pre-poured blood agar (BA, Difco, USA) plates (e.g. 0.4, 0.3 and 0.3 mL) and spread over the agar surface, incubated at 35 °C for 45–48 h (U.S. Food and Drug Administration, 1998). Colonies with 2–3 mm diameter, smooth convex, with gray to black color surrounded by an opaque zone were counted. Typical colonies were tested for coagulase production. The number of typical colonies on triplicate plates giving positive coagulase multiplied by the dilution factor was reported as *S. aureus*.g^{-1} sample.

Isolation and identification of histamine-forming bacteria

Histamine-forming bacteria were isolated by adding approximately 20 mL Niven's medium into diluted samples (1 mL). The plates were incubated at 37 °C for 24 h and reported as $\log_{10}$ colony forming units (CFU) g^{-1} of the dried fish sample. Niven's medium contained: tryptone (Difco, USA), 5 g; yeast extract (HiMedia, India), 5 g; L-histidine (Sigma, USA), 27 g; NaCl, 5 g; calcium carbonate ($CaCO_3$), 1 g; bacteriological agar (HiMedia, India), 20 g; and bromocresol purple (Sigma, USA), 0.06 g in 1 L distilled water and sterilized at 115 °C for 10 min (Niven et al. 1981). Colonies with evidently different morphological appearance (such as shape, elevation, surface and margin) were streaked separately on nutrient agar (HiMedia, India) plates to obtain pure culture.

Morphological characteristics such as colony morphology, Gram stain reaction and spore formation; and biochemical characteristics such as oxidase, catalase and other conventional biochemical tests as described in Bergey's Manual of Systematic Bacteriology (1957) were used to presumptively identify the seven isolates up to the species level. HighChrome Vibrio agar (Difco, USA) was used to differentiate *Vibrio* species, while Pseudo F and P agar (Difco, USA) were used to differentiate fluorescein and pyocyanin pigments, respectively in *Pseudomonas* species.

Histamine content analysis

Histamine content was analysed using flourometric method (AOAC 2012) with modifications. Briefly, a 5 g sample was transferred into 50 mL polypropylene tubes and homogenised with 40 mL methanol for 1 min. The homogenates were placed in water bath at 60 °C for 15 min, cooled at room temperature, then transferred in volumetric flasks and methanol was added to the final volume of 50 mL. The homogenates were then filtered using Whatman No. 1 filter paper (Whatman, Maid-stone, England). Using column chromatography, about 1 mL of sample extract was passed into a glass column containing an ion exchange resin at a flow rate of >3 mL.min^{-1}. Water was added on top of the resin until the eluate reaches 50 mL. The extract was subjected to fluorometric (Trilogy® Turner, USA) reading by pipetting 5 mL of sample into a 50 mL container. This was followed by the addition of 10 mL of 0.1N HCl and 3 mL of 1N sodium hydroxide (NaOH, Sigma, USA). Within 5 min of extraction, 1 mL of 0.1 % *o*-phthalaldehyde (OPA) (Sigma, USA) solution and 3 mL of 3.57 N phosphoric acid (H_3PO_4, Sigma, USA) were added. The fluorescence intensity was recorded during the 1.5 h at an excitation wavelength of 450 nm. Histamine content was expressed in mg.100 g^{-1}.

Statistical analysis

Data obtained were subjected to one-way analysis of variance and expressed as the mean ± standard deviation. Pearson correlation was performed to determine the relationships between pH, salt content, moisture content, Aw, TVB-N, and histamine content in the 21 dried fish samples. All statistical analyses were performed using the Statistical Package for Social Sciences, SPSS Version 16.0 for Windows (SPSS Inc., USA). Value of $P < 0.05$ was used to indicate significant deviation.

Results

Physico-chemical quality and histamine content of dried-salted fish samples

Range values of pH, salt content, moisture content, water activity (Aw), total volatile basic nitrogen (TVBN), and histamine content of the 21 dried fish samples from the retail markets in Iloilo province are shown in Table 1. For all the samples tested, the level of pH ranged 6.36–6.71; salt content, 2.55–15.84 %; moisture content, 21.12–46.41 %; Aw, 0.67–0.87; TVBN, 19.25–59.94 mg.100 g^{-1}, and histamine 0.42–60.75 mg.100 g^{-1}.

Table 1. The values of pH, salt content, moisture content, water activity, total volatile basic nitrogen and histamine in 21 major dried fish products.

Species	No. of samples	pH	Salt content (%)	Moisture content (%)	Aw	TVB-N (mg.100 g^{-1})	Histamine (mg.100 g^{-1})
Mackerel	7	6.36–6.51	7.39–15.84	42.07–45.31	0.75–0.87	19.25–59.94	0.42–17.37
(*Rastrelliger kanagurta*)		(6.46 ± 0.09)*A	(12.01 ± 3.33) A	(44.13 ± 1.25) A	(0.79 ± 0.04) A	(39.60 ± 20.85) A	(12.00 ± 12.65) A
Sardines	7	6.53–6.71	8.90–14.46	38.85–46.41	0.75–0.80	26.42–55.37	6.29–60.75
(*Sardinella* spp.)		(6.23 ± 0.07) B	(12.01 ± 1.94) A	(43.10 ± 2.69) A	(0.77 ± 0.01) A	(42.06 ± 13.92) B	(27.08 ± 20.67) C
Anchovies	7	6.36–6.63	2.55–8.41	21.12–27.06	0.67–0.78	36.96–50.69	2.61–30.54
(*Stolephorus* spp.)		(6.51 ± 0.10) A	(5.52 ± 2.38) B	(24.09 ± 2.00) B	(0.73 ± 0.04) B	(43.13 ± 5.58) B	(18.28 ± 10.09) B

Aw, water activity; TVB-N = total volatile basic nitrogen. *The values in the parentheses indicate the mean ± the standard deviation. The different capital letters (A, B and C) in the same column indicate a statistically significant difference ($P < 0.05$) between the means.

The average pH level (6.23) was significantly lower in dried-salted sardine samples than in mackerel and anchovies. While a significantly lower average salt content (5.52 %), moisture content (24.09 %) and Aw were found in anchovies ($P < 0.05$) and in mackerel samples, average TVBN (39.60 mg.100 g^{-1}) and histamine (12.0 mg.100 g^{-1}) were significantly lowest ($P < 0.05$). Pearson correlation was conducted to determine if any relationship existed among the pH, salt content, moisture content, Aw, TVB-N, and histamine of the 21 dried fish samples tested. In general, positive correlations were noted between moisture content and Aw ($r = 0.81$, $P < 0.05$), pH and TVB-N ($r = 0.88$, $P < 0.01$), histamine and TVB-N ($r = 0.80$, $P < 0.01$), and histamine and Aw ($r = 0.91$, $P < 0.01$). While negative correlations existed between pH and salt content ($r = -0.96$, $P < 0.01$), histamine and salt content ($r = -0.72$, $P < 0.05$) and salt and Aw ($r = -0.82$, $P < 0.05$). Distribution of histamine contents for dried-salted fish products were presented in Table 2, with only four samples (19.1 %) having histamine levels of <5 mg.100 g^{-1} which is the limit that is safe for consumption as suggested by Shalaby (1996). Sixteen out of 21 samples (76.2 %) contained histamine levels greater than 5 mg.100 g^{-1}, the allowable limit of the US Food and Drug Administration (FDA 1995) for scombroid fish and/or products. One sample had more than 50 mg.100 g^{-1} of histamine, the toxic level as per guidelines of US FDA.

Table 2. Distribution of the histamine contents in 21 dried fish products.

Content of histamine (mg.100 g^{-1})	No. of samples	% of samples
<4.9	4	19.1
5.0 – 19.9	10	47.6
20.0 – 49.9	6	28.6
>50	1	4.8
Total	21	100

Microbiological quality of dried fish samples and isolation of histamine-forming bacteria

The microbiological quality of the dried fish products is presented in Table 3. The percentage of unacceptable dried fish samples was 52.4 % (11/21) for APC, based on the Philippine regulatory standard of 5.69 log CFU.g^{-1} (5×10^5 CFU.g^{-1}). Significantly higher ($P < 0.05$) average APC (6.37 log CFU.g^{-1}) were detected in dried anchovies samples than in dried mackerel (5.81 log CFU.g^{-1}) and sardines (5.81 log CFU.g^{-1}). For *S. aureus* count, most of the samples exceeded the *S. aureus* limit of 3.0 log CFU.g^{-1} (1×10^3 CFU.g^{-1}) set by the Bureau of Food and Drug-Philippine National Standards (BFAD-PNS, 2006) with dried anchovies samples having significantly highest average *S. aureus* count of 5.03 log CFU.g^{-1} ($P < 0.05$). Results for TCs and *E. coli* were <3 MPN.g^{-1} for all dried-salted fish samples tested. These values were below the standard limits of TC and *E. coli* which are 100 MPN.g^{-1} and 11 MPN.g^{-1}, respectively, set by BFAD-PNS. Results also show that *S. typhi* was absent in 25 g for all samples tested while HFB count for dried mackerel samples was 4.34 log CFU.g^{-1} and none were detected for dried sardines and anchovies.

Table 3. Microbiological quality of 21 major dried fish products.

Species	No. of samples	APC (log CFU.g^{-1})	*S. aureus* (log CFU.g^{-1})	TCs (MPN.g^{-1})	*E.coli* (MPN.g^{-1})	*S. typhi* (+/-) in 25 g	HFB (log CFU.g^{-1})
Mackerel	7	4.66–6.82	3.0–4.50	<3	<3	Absent	4.34
(*Rastrelliger kanagurta*)		(5.81 ± 0.80)*A	(3.72 ± 0.75) A				
Sardines	7	3.0–7.36	3.59–4.39	<3	<3	Absent	<1
(*Sardinella* spp.)		(5.81 ± 0.88) A	(3.94 ± 0.41) A				
Anchovies	7	5.0–7.39	3.84–5.63	<3	<3	Absent	<1
(*Stolephorus* spp.)		(6.37 ± 0.71) B	(5.03 ± 1.03) B				

APC = aerobic plate count; CFU = colony forming unit; *E. coli* = *Escherichia coli;* HFB = histamine-forming bacteria*;* MPN = most probable number*; S. aureus* = *Staphylococcus aureus*; *S. typhi* = *Salmonella typhi*; TCs = total coliforms. *The values in the parentheses indicate the mean ± the standard deviation. The different capital letters (A, B and C) in the same column indicate a statistically significant difference ($P < 0.05$) between the means.

Table 4. Biochemical and morphological test results for the presumptive identification of histidine-decarboxylating bacterial (HDB) isolates from the dried fish samples.

Biochemical test	Histidine-decarboxylating bacterial (HDB) isolates						
	DM4	MM7	DR5	RD3	MM6	RM1	RM9
Gram staining	-	-	-	+	+	-	-
Shape	Rod	Rod	Rod	Cocci	Rod	Rod	Rod
Arrangement	Single, comma shape	Single, comma shape	Single, small rods	Pairs, short chains	single, others chain	Single, small	Single, small
Spore staining	-	-	-	-	+	-	-
Catalase	+	+	+	+	-	+	-
Starch hydrolysis	+	-	+	+	-	+	+
Casein hydrolysis	+	+	+	+	+	-	+
Citrate utilization	+	+	+	+	-	-	-
Indole	-	-	-	-	-	-	-
Methyl red	-	+	-	-	-	-	-
Vogues Proskauer	+	-	+	+	-	-	-
Oxidase	+	+	-	-	+	+	-
Acid fermentation:							
Glucose	+	+	+	+	-	-	-
Mannitol	+	+	+	+	-	-	-
Lactose	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-
Motility	-	-	+	-	-	-	-
Pseudo F agar	-	-	-	-	-	+	+
Pseudo P agar	-	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-	-
Lecithinase	-	-	-	-	-	-	-
HighChrome agar	white	Blue-green					
Presumptive Species	*Vibrio alginolyticus*	*Vibrio parahaemolyticus*	*Salmonella enterica*	*Staphylococcus aureus*	*Bacillus* sp.	*Pseudomonas putida*	*Pseudomonas syringae*

+ = positive result; - = negative result

Seven species of bacteria were isolated and identified to be histamine formers in dried mackerel samples. As shown in Table 4, these were found to belong to genus *Vibrio, Salmonella Staphylococcus, Bacillus,* and *Pseudomonas.* The histidine-decarboxylating bacterial (HDB) isolates belonging to *Vibrio* species were DM4 and MM7 presumptively identified as *Vibrio alginolyticus* which produced white colonies on HighChrome agar and *Vibrio parahaemolyticus* with blue-green colonies, respectively. Two HDB isolates namely RM1 and RM9 belonged to *Pseudomonas* species and were presumptively identified as *Pseudomonas putida* and *Pseudomonas syringae*, respectively. Both strains gave a negative reaction to nitrate reduction and exhibited fluorescence through fluorescein production in Pseudo F agar, typical for both *Pseudomonas* species. Isolate RM1 was lecithinase negative, the identifying characteristic of *Pseudomonas putida*, while isolate RM9 was oxidase negative, which is typical of *Pseudomonas syringae*. Other HDB isolates were DR5, RD3 and MM6 which were presumptively identified as *Salmonella enterica*, *Staphylococcus aureus* and *Bacillus* sp., respectively.

Discussion

The physico-chemical quality of traditionally dried-salted fish products (Table 1) was generally inferior as compared to the standards. The resultant pH values in all the samples are in a mild acidic condition which is in agreement with the previous reports of Kuda et al. (2007) and Tsai et al. (2006) in salted and fermented fish products with pH range values of 4.95–6.54. The initial decrease in muscle pH at early postmortem may be due to the decomposition of glycogen in whole fish. While the increase in pH at the later stages of storage could be attributed to the production of volatile basic compounds, such as ammonia and amines by fish spoilage microbial action and autolytic reactions (Huss 1988; Farid et al. 2014). For the salt content, 28.6 % (6/21) of the dried fish samples complied with the suggested level of not less than 12 % salt content for dried fish products set by the BFAD-PNS (2006).

The low salt content of the majority of the commercial dried products may favor bacterial proliferations which are precursors for the synthesis of histidine decarboxylase to convert histidine to histamine in the fish muscle (Eitenmiller et al. 1982; Taylor et al. 1989). High moisture contents were observed on dried mackerel and sardine samples which could be attributed to the inadequate drying during the processing of the dried products. The moisture content of normally prepared freshly dried fish product is around 22 % (Kalaimani et al. 1988). Values higher than 22 % cannot prevent the degradative activity by enzymes and microorganisms in advance spoilage processes, since high moist foods are unstable and prone to fly infestation (Doe et al. 1982). Moreover, an observation was made that fish processors sometimes allow more moisture in dried fish products to gain weight for economic benefit. The other reason is that the dried products used for selling in both the wholesale and retail markets as well as during storage normally do not use a suitable packaging material. In a tropical country like the Philippines where relative humidity is always high, excessive moisture uptake increases the water activity (Aw) which facilitates the growth of microorganisms and reduces the shelf life of dried products.

Aw gives potential effect on the stability of food products against the chemical reaction and microbiological growth rate in low and intermediate-moisture foods (Labuza 1980; Roos et al. 1996). Ten samples (47.6 %), mostly from dried mackerel and sardines, were found to exceed the standard 0.78 Aw limit set by BFAD-PNS (2006) for dried-salted fish. The risk of pathogenic and histamine-forming bacterial activity is high in processed fish products that are salted and dried with an Aw value above 0.80 (Kose 2010). Furthermore, 85.7 % (18/21) of the dried fish samples were unacceptable for TVBN, based on the decomposition limit level of 30 mg.100 g^{-1} for fish quality determination (Kung et al. 2015). The increase in TVBN, which is primarily composed of ammonia and primary, secondary and tertiary amines, is related to the accumulation of spoilage bacteria and endogenous enzymes in fish (Doe et al. 1982). Tsai et al. (2012) implicated that increase in TVBN levels in dried fish products can be due to favorable temperature (30–35 °C) for enzyme activity and bacterial growth. In addition, the low salt content strongly supports the survival of ammonia-producing bacteria and spoilage bacteria in dried-salted products even at high temperature because of the lack of preservative action of salt.

Legal limits of histamine levels that are regarded as safe for human consumption were set by various countries: Australia, 20 mg.100 g^{-1} (Australian Food Standards Code, 2001), Europe, 10 mg.100 g^{-1} (EC, 2003) and South Africa, 10mg.100 g^{-1} (South African Bureau of Standards, 2001). But a more rigid FDA level of 5 mg.100 g^{-1} as an indicator of decomposition in dried fish was applied in this study. In fact, one dried sardine sample in the present study could have caused disease symptoms when consumed, mainly because of the high histamine content (60.75 ± 0.04 mg.100g^{-1}) of greater than 50 mg.100 g^{-1} and 16 dried-salted fish samples were possibly toxic having histamine levels greater than 5 mg.100 g^{-1} (Table 2). Several studies confirmed high histamine levels in many kinds of fish specifically in sardines, mackerel and anchovies implicated in scombroid poisoning.

The presence of histamine-producing bacteria in sardines stored at ambient temperature was confirmed by Ababouch et al. (1991), while an increased histamine level of 100 mg.100 g^{-1} was observed by Yatsunami and Echigo (1991) on a histamine producer bacterial strain isolated from a commercial rice-bran pickle of sardines. Arnold and Brown (1978) showed the increase in histamine level during handling and salting process of split dried-salted mackerel and Tsai et al. (2005) also reported that two of the eighteen commercial samples of salted mackerel exceeded the 50 mg.100 g^{-1} defect action limit. Further, Hernandez-Herrero et al. (1999) detected 68 mg.100 g^{-1} histamine in semi-preserved Spanish anchovies involved in a case of histamine poisoning. High levels of histamine were also found in freshwater fish such as catla *Catla catla* (Hamilton, 1822) and rohu *Labeo rohita* (Hamilton, 1822) where it exceeded the maximum limit of acceptability of 20 mg % after 18 h of storage at 30 °C and 5 days of storage at 5 °C (Jeya Shakila and Vasundhara 2002). Thus, it is necessary to be cautious that dried fish could be a potentially hazardous food item in causing histamine poisoning. Proper and hygienic fish processing procedures during cutting, salting, drying and packing of these dried products should inhibit histamine-forming bacteria and reduce the histamine level. Majority of dried-salted fish samples exceeded the acceptable limit for APC and *S. aureus* count as set by the BFAD-PNS (2006).

Although TC, *E. coli* and *S. typhi* counts were negligible, one dried mackerel sample had counts of histamine-forming bacteria isolated in the Niven's medium (4.34 log CFU.g^{-1}). The results obtained in this study coincided with previous studies on potential histamine-forming bacteria, including halotolerant bacteria isolated from various salted fish samples. For instance, *Staphylococcus* spp., *Vibrio* spp. and *Pseudomonas* III/IV-NH were isolated from fermented salted sardine products (Yatsunami and Echigo 1991, 1992) while *Staphylococcus epidermidis, Staphylococcus xylosus, Klebsiella oxytoca, Enterobacter cloacae, Pseudomonas cepaciae,* and *Bacillus* spp. were isolated from salted anchovies (Rodriguez-Jerez et al. 1994; Lopez-Sabater et al. 1996). *Bacillus, Staphylococcus* and *Micrococcus* were also the major genera isolated from fishmeal and shrimp feed marketed in India found to contain high levels of histamine (Kennedy et al. 2004).

A weak histamine former belonging to *Salmonella enterica* was also isolated in this study, which was confirmed by Huang et al. (2010). Their study isolated strains of *Salmonella* from Pacific round herring *Etrumeus teres* (DeKay 1842) and blue-spotted stingray *Dasyatis kuhlii* (Müller and Henle 1841). Moreover, *Staphylococcus* spp. were demonstrated to have powerful histamine-forming activity and accounts for around 50 % of histamine-forming microorganisms (Yatsunami and Echigo 1991, 1992). Specific strains of *Staphylococcus* namely *S. epidermidis* and *S. capitis* isolated from salted Spanish anchovies, produced more than 1000 ppm and 400 ppm of histamine, respectively, in TSBH broth (Hernandez-Herrero et al. 1999). Given that *Staphylococci* are the major microflora of the human skin, it is reasonable to expect that they would be transferred to food products through considerable human contact during preparation and processing.

Histamine-forming bacteria can possibly penetrate from the intestine into the inner muscle of whole fish during the sun-drying process by rupture of the belly walls (Moori et al. 1988). In addition, cross-contamination may occur as the fish is eviscerated during manual processing, cut with a dirty knife, and mixed in the same tank with the brine for a prolonged time. Also, dried fish samples were stored at room temperature during and after sun-drying where flies may easily contaminate. These may be the reasons that these histamine-forming bacterial strains are present in the dried-salted fish products.

Conclusion

Results revealed that 16 out of 21 (76.2 %) tested dried fish products had histamine levels greater than the FDA guideline of 5 mg.100 g^{-1} for scombroid fish and/or products. Highest histamine content of more than 50 mg.100 g^{-1} was detected in one dried sardine sample. Consumption of these dried products could exacerbate histamine poisoning in susceptible individuals.

Microbiological quality also indicated that aerobic plate count and *S. aureus* count exceeded the standard limits set by the Philippine regulatory standards. Seven species of bacteria were identified as histamine formers. Therefore, aside from histamine, the hygienic quality of traditional dried-salted fish products needs to be improved.

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